Studies of the biology of the trematode brachylaemus

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STUDIES ON THE BIOLOGY OF THE TREMATODE BRACHYLAEMUS.

by

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CONTENTS.

I. INTRODUCTION AND HISTORICAL REVIEW.

(a) The Problem.

Probably because of their usually encysted condition, the metacercarial stages in trematode life-histories are supposed to have little or no effect on their hosts (Brown 1926; Hurst 1927). As this assumption has been very widespread, very little investigation to determine whether or not some trematode metacercariae may in fact exert a considerable effect on their hosts, has been done.

Cysts do not occur around the metacercariae of some species of the family Brachylaemidae. Joyeux and Foley 1930, while other species of the family possess only a thin, jelly-like membrane in occasional individuals, which is not regarded as a true cyst. (Ulmer 1951).

The discovery at Durham in 1949 of completely unencysted metacercariae in slugs (Cragg and Vincent, unpublished) enabled an investigation to be made not only into the nature and ecology of such metacercariae, but also into any effects which they might exert on the life and activity of their hosts. This thesis contains the result of that investigation.

As will be shown later, the taxonomic state of the family Brachylaemidae is most unsatisfactory.

Further it has not been possible to produce an adult fluke from the present metacercariae. From the larval characters which have been observed, however, the present form appears to be of the genus Brachylaemus (Dujardin 1843) Blanchard 1847, but it is impossible to identify it specifically (Dollfus, personal communication) It is unlike any previously described form.

Table 1 gives a comparison of the observed characters with those agreed upon for the genus Brachylaemus; the latter characters are taken from several authors, but mainly from Dawes (1946).

In the Table, the terminology at X is that of Dawes (1946), and not that used in section II (p.34). Although the structures are similar, the terminology employed on P.34 is preferred to that of other authors because of its simplicity.

Pavlovskaia (1953) and Dollfus (personal communication, 1954) have both commented on the wide variability of individual brachylaemids, and the subsequent difficulty of ascertaining representative characters. The latter author considers specific identifications to be impossible in the majority of cases.

TABLE I

Observed Characters and Characters of Genus.

Agreed characters of Genus Brachylaemus.	Observed characters of Metacercariae.
1. Length of body 1 - 7 mm.	1. Length of body approx. 3 mm.
2. Elongate oval - elongate flattened.	2. Elongate oval.
3. Cuticle smooth or spiny.	3. Cuticle smooth, but wrinkled during one developmental stage.
x 4. Prepharynx, pharynx and oesophagus present. Oesophagus short	x 4. Prepharynx, pharynx, and oesophagus present. Oesophagus short.
5. Ventral sucker equatorial.	5. Ventral sucker equatorial.
6. No additional adhesive organ or gonotyle.	6. No additional adhesive organ or gonotyle.
7. Gut caecae reaching almost to anterior limit.	7. Gut caecae reaching almost to anterior limit
8. Genital pore far back.	8. Genital pore 1/3 to ¼ distance behind ventral sucker.
9. Cirrus pouch small or absent.	9. Unknown.
10. Testes rounded, one behind other.	10. Unknown.
11. Ovary between testes, rounded and median.	11. Probably median.
12. Uterus with ascending and descending limbs. Extends in front of ventral sucker.	12. Unknown.

TABLE I (contd).

13. Eggs small, usually, 0.02 - 0.03 mm. in length.	13. Unknown.
14. Laurer's Canal present.	14. Unknown.
15. Receptaculum seminalis usually absent.	15. Unknown.
16. Vitellaria well-developed, but extend posteriorly only to anterior testis.	16. Unknown.
17. Excretory vesicle Y - shaped. Tubules extend to anterior limit of body. Ciliated ducts present.	17. Excretory vesicle Y - shaped. Tubules extend to anterior limit of body. Ciliated ducts present.
18. Metacercariae possess caudal appendages when young.	18. Caudal appendages present for first 4 - 5 weeks of life.

(b) The Taxonomic History of the family Brachylaemidae and the genus Brachylaemus.

Owing to the inadequacy of the original description of the genus Brachylaima given by Dujardin (1843), the history of the family Brachylaemidae Joyeux and Foley 1930 (= Harmostomidae Odhner 1912) has been confused from its inception.

Dujardin (1843) created the genus Brachylaima for a trematode advena from the shrew Sorex leucodon Zimmermann, and recorded it as identical with a distome said to arise "spontaneously" in the liver of nearby limacids. He suggested that the parasites had to be transferred into a mammal before eggs could be prpduced, and considered that his work proved ".... le transmigration des Helminthes." He designated B. advena as type, but described it very poorly, giving the main character as ".... l'extreme briéveté de son oesophagien." He described other "brachylaemids" (since transferred to other genera) from fish (Pleuronectes solea L.) and amphibians ("Grenouille rousse").

Two years later (1845), Dujardin reviewed the genus, but referred to it as a subgenus of the genus Distomum, and changed the spelling to Brachylaimus. He listed 20 species in five separate groups distinguished on the relative positions of the genital organs, and described Distoma migrans from Sorex araneus L. and Sorex Leucodon Zimm. fairly fully.

Stiles and Hassall (1898) showed that his reference to "var∝ of D. migrans" was a renaming of the type-species B. advena, described two years previously. He had noted, however that he found it impossible to distinguish this form from others found in neighbouring molluscs. In the descriptions of these larval forms in molluscs, he noted the presence of "vibrating cilia" but recorded that no genital organs were visible.

Blanchard (1847) claimed that the subgeneric name was improperly formed, and corrected it to Brachylaemus, simultaneously restoring it to full generic rank (reasons not evident). It thus became Brachylaemus (Dujardin 1843) Blanchard 1847. Blanchard listed as types B. cylindraceus and B. variegatus, both from frogs. Neither of these species were mentioned by Dujardin (1843), and both have since been re-assigned to other genera. The former is now Haplometra cylindracea (metacercariae in insects), and the latter Haematoloechus variegatus. A third species, B. erinacei, was later transferred to the genus Mesogonimus. He further noted that the oesophageal character of Dujardin was insufficient to be diagnostic, and called for a review of general body form and structure.

Stiles and Hassall (1898) disagreed with the orthographic correction of Blanchard (1847), retaining the generic name of Brachylaima. Further reference to the name will be made later. They also observed that

"..... the fate of the genus Brachylaima depends upon the correctness of the anatomical descriptions given by Dujardin." They complained of the inadequacy of the original descriptions, and indicated the need for more thorough work, with special reference to the position of the genital openings.

A re-examination of the species was made by Baer (1928) whose findings were based on a comparison of Dujardin's specimens with others collected from shrews near Champéry (Valois). He concluded that although B. advena had priority over B. migrans, the description of the former was too brief to be valid; the type-species thus became B. migrans.

Meanwhile the generic name Brachylaemus was itself falling into disuse due to the erection by Braun (1899) of the genus Harmostomum. In this new genus the genital pore was situated between the anterior testis and the ventral sucker. Braun designated Distomum leptostomum Olss. 1876 as type, and listed a number of other species, noting that D. recurvum Duj. 1845 and D. migrans Duj. 1845 might be included.

The same year, Looss (1899), using the same characters as Braun, established the genus Heterolope, again with D. leptostomum Olss. 1876 as type. He further erected the subfamily Heterolopinae (Family Fasciolidae) to include the genera Heterolope Looss 1899, Harmostomum Braun 1899,

and the species Distomum lorum Duj. 1845.

Braun (1901) claimed that as the genera Harmostomum and Heterolope were obviously synonyms, and because the name Harmostomum was older than Heterolope, the subfamily Heterolopinae Looss 1899 should have been Harmostominae Braun 1899. This was accepted, and as further species were discovered, was followed by the erection of the family Harmostomidae Odhner 1912) and the tribe Harmostomea (Witenberg 1925).

Joyeux and Foley (1930) however concluded that Harmostomum Braun 1899 was itself a synonym of Brachylaemus (Dujardin 1843) Blanchard 1847. Consequently all the superior names based on Harmostomum as a genus fell with the generic name, and the revised names for the family, subfamily, and tribe became Brachylaemidae, Brachylaeminae, and Brachylaemea. respectively.

After studying the International Rules of Nomenclature, certain recent authors, particularly Odlaug (1951), have reverted to the original spelling of the generic name Brachylaima, as did Stiles and Hassall (1898). Dollfus (1954 - personal communication), however, expressed the opinion that the correct romanized derivation of the Greek roots was Brachylaema.

The present author makes no decision regarding the correct nomenclature of the genus, but continues to use Brachylaemus, as the commonest form in modern usage.

The various names still met with in the literature are summarized below:

Family Brachylaemidae Joyeux and Foley 1930
(= Harmostomidae Odhner 1912)

Subfamily Brachylaeminae Joyeux and Foley 1930
(= Harmostominae Braun 1899)
(= Heterolopinae Looss 1899)

Genus Brachylaemus (Dujardin 1843) Blanchard 1847
or Brachylaima Dujardin 1843
or Brachylaema Dujardin 1843
(= Harmostomum Braun 1899)
(= Heterelope Looss 1899)

Type-species B.migrans Dujardin 1845

The first real attempt at a classification of the group appears to be that of Witenberg (1925). Although primarily concerned with the genus (then subgenus) Postharmostomum Witenberg 1923, he outlined a classification of the Harmostominae, erecting four new tribes. These, with their respective genera, were tribe Ithyogonimea Witenberg 1925 (Ithyogonimus Lühe 1899, Scaphiostomum Braun 1901); tribe Harmostomea Witenberg 1925 (Harmostomum Braun 1899, with two subgenera Harmostomum Witenberg 1925 and Postharmostomum Witenberg 1923, Glaphyrostomum Braun 1901); tribe Urotocea

Witenberg 1925 (Urotocus Looss 1899); and tribe Leucochloridiaea Witenberg 1925 (Leucochloridium Carus 1835).

Sinitsin (1931) proposed an entirely new scheme of the family Harmostomidae, dividing it into two tribes, Ectosiphonea and Entosiphonea, on the basis of the position of the main excretory ducts in relation to the intestinal crura. In the Entosiphonea he included spinose Harmostominae with the two main siphons of the excretory bladder situated intracecally; in the Ectosiphonea he included aspinose Harmostominae whose main excretory ducts were extracecal. He erected several new genera, including in the tribe Entosiphonea the genera Harmostomum Braun 1899, Postharmostomum Witenberg 1923, Urotocus Looss 1899, Hasstilesia Hall 1916, and Entosiphonus Sinitsin 1931. In the tribe Ectosiphonea he included the genera Ithyogonimus Lühe 1899, Scaphiostomum Braun 1901, Panopistus Sinitsin 1931, and Ectosiphonus Sinitsin 1931.

The validity of Sinitsin's division of the family into these two tribes was questioned by Adam and Leloup (1934), who pointed out that within a single species the excretory ducts may vary in position, especially after fixation. In the species described in this thesis, the siphons have certainly been seen both intracecally and extracecally "in vivo". There is now general agreement that Sinitsin's division was invalid.

Dollfus (1934) accepted the views of Joyeux and Foley (1930) on the synonymy of Harmostomidae and Brachylaemidae, and revised the family. With certainty, only the genera Brachylaemus (Duj. 1843) Blanchard 1847 and Itygonimus Baer 1932 (= Ithyogonimus Lühe 1899) were retained. The genera Leucochloridium Carus 1835 (= Urogonimus Monticelli 1888) and Urotocus Looss 1899 were formed into a new family, the Leucochloridiidae (= Urogoniminae Looss 1899) (= Leucochloridiinae Poche 1907). Other genera were also removed to other established or newly established families.

Mehra (1936) considered Brachylaemidae Joyeux and Foley 1930 to be a synonym of Harmostomidae Odhner 1912, but his reasons were not evident. He further alloted the genera of the Harmostomidae (including those rejected by Dollfus 1934) into six subfamilies designated Harmostominae Braun 1899, Hasstilesiinae Hall 1916, Leucochloridiinae Poche 1907, Liolopinae Cohn 1902, Harmotrematinae Yamaguti 1933, and Morgauiinae Johnston 1915, distinguishing them on the shape and form of body, vesicular seminalis, uterus, vitellariae, and the position of the genital pore. A more recent taxonomic attempt was that of Allison (1943). Taking Leucochloridiomorpha constantiae as representative of the brachylaemids, he compared the family with other trematode groups from the point of view of the structure and development of all stages in the life-history. He

concluded that the Brachylaemidae were genetically related to the Strigeatoidea La Rue 1926, and " ... as the family does not fit into any of the established superfamiles, it must be given rank coordinate with them." The family was thus removed from the Prosostomata and placed in the order Strigeatoidea La Rue, a new superfamily, Brachylaemoidea, being erected with Brachylaemidae as the type-family. Allison further considered that the six subfamilies of Mehra (1936) (p.11) fell into two groups on the basis of size and number of eggs, position of genital pore, extent of vitellaria, size and position of vesicular seminalis, and vertebrate hosts. One group, the Brachylaeminae (birds and mammals), Leucochloridiinae (birds and mammals), and Hasstilesiinae (mammals), was retained as the family Brachylaemidae. The members of the other group, Moreauiinae (monotremes), Liolopinae (amphibians), and Harmotrematinae (reptiles), were each raised to family rank. He observed that "... further attempts to determine a more precise relationship of the sub-families of the Brachylaemidae inter-se, and of these with other members of the Strigeatoidea must await the solution of many more life-histories, and should include studies of the development of reproductive organs and especially of the excretory system."

Ulmer (1951) presented an emended diagnosis of the subfamily Brachylaeminae, noting that ciliated excretory ducts may or may not be present, that the cirrus sac is small or absent, that the genital pore may be median to terminal posteriorly, and that the miracidial cilia are unequally distributed and may be on bars.

The present state of the family is obviously unsatisfactory; much of the confusion has arisen apparently from an inability to determine the significance of the observed characters. Thus widely divergent classifications have been based on the same observations, and many changes made without any reasons being given.

(c) The Biology of the genus Brachylaemus

Dujardin (1843) first suggested a possible brachylaemid life-history, when he commented on the similarity of B.advena from shrews to larval forms in nearby molluscs, and suggested a transference from the molluscs to the guts of mammals where development was completed.

The attempts of Ercolani (1882) to complete the life-history of brachylaemid cercariae and sporocysts from Helix carthusianella were unsuccessful.

The first recorded life-history for the family was when Heckert (1887, 1889) traced the development of Leucochloridium paradoxum from snails (Succinea sp.)

to various Sylviidae.

Blochmann (1892), on the basis of morphological similarity and uncontrolled experiments, assumed that larval stages of Brachylaemus helicis, which he found in hedgehogs, occurred in Helix hortensis.

Hofmann (1899) described the metacercariae of B.spinulosum from six species of snails, and also Arion sp. The adults he found in Erinaceus europeus.

Magath (1920) in recording sporocysts of Leucochloridium problematicum from Planorbis trivolvis and Succinea retusa, observed that they resembled adults of Leucochloridium insignis and L.ceratum, and suggested that they may be the same species.

Ozaki (1925) recorded metacercariae of Harmostomum horizawai (= Brachylaemus commutatum Dies 1858. var. gallina Witenberg 1923) in the pericardium of Eulota peliomphala and Phylomycus bilineatus in Japan. The adults were in fowls.

Sinitsin (1931) described many brachylaemid life-histories, basing his conclusions only on the morphological similarity of larval and adult forms. His findings were regarded as not conclusive by Krull (1935).

Stiles and Stanley (1932) reported that adults of Harmostomum dujardini Baer 1928 (= Distoma migrans Duj. 1845) were found in Sorex araneus, and that the metacercariae "... seem to be in Agriolimax (agrestis)." Vectors listed for larval brachylaemids included

Arianta arbustorum, Cepaea hortensis, Cepaea nemoralis, Euomphalia strigella, Helix pomatia, Succinea sp., and Arion sp. Adults were found in Erinaceus sp., Talpa sp. and Sorex sp.

Joyeux, Baer, & Timon-David (1932, 1934) described the life-history of Brachylaemus fuscatus Rud. (= B. nicolli Witenberg), in which Passer domesticus and Turdus merula were infected by metacercariae from Helicella scitulla, Oxychilus cellaris, and Agriolimax agrestis.

Dollfus (1934, 1935) published an account of the brachylaemids of the French fauna. He listed some 25 species (with synonyms) together with hosts and world geographical distribution.

Dollfus (1935) produced a catalogue of hosts, and descriptions of a number of distomes from terrestrial Stylommatophora (excluding Succineidae)

Strix aluco and Tyto alba (Strigiformes) were infected with unnamed larval brachylaemids from Arion rufus by Dollfus, Callot, and Desportes (1935).

Krull (1934, 1935, 1936) described the life-history of B.virginiana (Dickerson) Krull by establishing adults in opossum (natural host), dog, cat, white-rat, and chicken (experimental hosts), and larvae in Polygyra thyroides (natural host,) Helix pomatia and Deroceros laeve (land snails), and Pseudosuccinea columella, Helisoma trivolvis, and Succinea sp. (aquatic snails).

Balozet (1936, 1937) completed the life-history of B.suis by feeding metacercariae from Xerophila sp.

and Rumina decollata to pig (natural host), rabbit, rat, mouse, and pigeon (experimental hosts). He further recorded the "...rôle pathogène" of the adult in the pig.

Alicata (1938, 1940) in Hawaii reared metacercariae from various land snails to adults in the ceca of chickens (natural hosts)

Reynolds (1938) recorded three metacercariae from Agriolimax agrestis; he suggested they were B.virginiana, but did not state whether they were encysted. He also recorded a new species B.peromysci, from the deer-mouse; in form it resembled B.virginiana.

Dollfus (1938) recorded a brachylaemid metacercaria from renal tissue in Helicella obvia in Bulgaria, designating it a member of the group "Brachylaemus caudatus"; attempts to produce adults in rats were unsuccessful.

Harkema (1939) recorded B. mcintoshi n. sp. from the ileum of the Barred Owl.

Da Fonseca (1939) described B.fleuryi n. sp. from hens.

Tubangui and Masilungan (1941), while working with Phillipine vertebrates, recorded B.malayensis in hens (cloaca), and observed that the size of the eggs, suckers, and extent of the uterus were characteristic.

Allison (1943) observed that in all known life-histories of the Brachylaeminae, the vertebrate host is a mammal or bird. Also, with the exception of

Leucochloridiomorpha constantiae, all known brachylaemid cercariae develop in terrestrial snails or slugs. He suggested that the group was originally to be found in aquatic snails, the cercariae having natatory tails. The latter had become reduced to small caudal appendages with the adoption of terrestrial molluscs as intermediate hosts. L.constantiae, he considered, by virtue of possessing both a natatory tail and an aquatic intermediate host (Campeloma decisum), was a "living ancestor" of the family Brachylaemidae.

McIntosh (1950) described Brachylaima rauschi n.sp. from the Arctic Lemming, reverting to the original spelling of the generic name (p.8). In this he was followed by Odlaug (1951) who described Brachylaima condylura n. sp. from the star-nosed mole.

The genus appears to be widespread both geographically and ecologically in birds and mammals. Under laboratory conditions, many species seem to show little specificity in their choice of hosts.

(d) The Effect of Larval Trematodes on their Hosts

Unlike the effects of adult flukes on vertebrate hosts, the effects of larvae on intermediate hosts are rarely of medical or veterinary significance; consequently the problems confronting the intermediate hosts have received but little attention from previous workers. Such work as has been done has been confined almost exclusively to the redial, sporocyst, and cercarial stages in the life-history.

Among the molluscan organs to suffer from such larval trematode infections are the liver and gonads. Rees (1936) reporting on the comparative effects of five separate species of cercariae on the tissues of Littorina littorea L., concluded that the effects varied with the peculiarities of each life-history, and the size of the parthenitae. If the infection was by rediae, and the sporocyst generation was lacking, the first organs to be attacked were the gonads. The attack was from the inter-lobular lymph areas of the liver, but the latter itself was affected only indirectly through loss of food, pressure, etc. If the initial attack was by a sporocyst however, and rediae were lacking, then the gonads were preserved much longer, and their ultimate disappearance was due to indirect causes such as pressure-atrophy and an accumulation of waste matter. If the parthenitae were non-migratory, a "blocking-layer" formed, isolating a part of the liver and leading ultimately to its death. Rees further recorded the invasion of the stomach and kidney by cercariae, observing that this caused " ... little or no physiological disturbance," but that a general degeneration of liver and gonadial tissues occurred during infection.

Degeneration of liver tubules during infection of Lymnaea natalensis by two larval flukes was recorded by

Fantham and Porter (1936), who suggested a transference of glycogen and protein from the host-cells to those of the parasites. A similar effect was noted in Stagnicola sp. by Pratt and Lindquist (1943), who thought that a suspension of secretory activity occurred during parasitism.

Pratt and Barton (1941) claimed that during infection of Stagnicola emarginata angulata by four species of larval trematodes, there was only a reduction in the number of liver tubules, and no actual cell-destruction. Agersborg (1924) had previously recorded atrophy of the nepatic epitheleum as larval trematodes migrated through the bodies of certain snails, noting tnat when the cytoplasm disappeared, the residual nuclei often became aggregated into a cyncitium. He also noted several distinct morphological differences in the liver of the same individual at the same time. Further, he showed that degeneration could be followed by regeneration, and a certain degree of tissue-recovery. In this he confirmed similar views expressed by Faust (1920) who studied the effects of parasitism on Planorbis guadelupensis, Physopsis africana, Planorbis trivolvis, and Physa sp. Faust noted that in addition to a general hepatic necrosis, there were also abnormal cell-divisions in the liver, normal mitosis being virtually absent. He also noted several secondary effects. The host was unable to prevent such undigested food and

faeces as normally pass through the gut from enterin, the damaged liver. Similarly, as the cilia of the intestinal epithelia failed to function, diatoms and algae with particles of silica also entered the liver tubules.

Faust (1920) further described dense agglomerates in the liver cells. These were possibly of complex mucoids, and ultimately disappeared. An increase in the acidity of the parasitized tissues was also recorded.

The effect on the gonads of the host has also been noted by several workers. Crewe (1947) claimed that castration resulted from helminth parasitization of limpets although the vitality of the host was unaffected. An increasing sterility and reduced reproductive rate during the parasitization of many marine molluscs was noted by Kuznetzov and Tchubrik (1950). Similar effects were also recorded during infection by Leucochloridium paradoxum (Brachylaemidae) by Wesenberg-Lund (1931), who further showed that the gonads may be redeveloped when the infection subsided. He pointed out that "sexual change-overs" have not been recorded as a result of parasitization by this trematode.

The true effects of the larvae on the gonads are not clear. Although "parasitic castration" is often claimed, Filhol (1938) said that this does not occur; he considered the chief action of sporocysts to be a

cutting off of the blood- and food-supply to the gonads, arresting their development in an embryonic state, or, in the case of older individuals, leading to atrophy. It is further possible that "reduced reproductive rates" are not always as straightforward as they appear. Barnes (1953) recorded that parasitization of Balanus balanus by a gregarine apparently reduced the reproductive rate of the host. Actually it affected not the reproduction, but merely the release of the larvae, the first-stage nauplii being retained within the parent. When released artificially, these larvae proved to be healthy, and changed immediately into second-stage nauplii.

During infection of Physa occidentalis by larvae of Echinostomum revolutum, relatively more (7.9%) carbon dioxide was eliminated, and relatively more (7.1%) oxygen consumed (Hurst 1927). The glycogen and reducing-sugar content often showed a decrease during infection, while the fat content was increased. Working on similar lines, von Brand and Files (1947) concluded that infection of Australorbis glabratus by Schistosoma mansoni did not seriously interfere with either fat-storage or oxygen consumption of the host. They found a reduced polysaccharide content and storage capacity however, which may have been due to impaired carbohydrate digestion and resorption, or to a toxic action.

Gigantism of Peringia ulvae infected by larval trematodes was noted by Rothschild M. (1936), Rothschild, A. & M. (1939), and Rothschild, M.(1941). The hosts had a greater final size after a more rapid growth, irregularities of the shell-whorls, and an assymmetry of the spire. Gigantism in Stagnicola sp. during infection was recorded by Pratt and Lindquist (1943)

Agersborg (1924) observed an increase in black granules throughout various snails parasitized by larval trematodes. He suggested that the granules were of nuclear origin, and served to counteract the toxins of the parasites. The suggestion was refuted by Hurst (1927) who claimed that the granules were unrelated to the parasitism.

The hosts kidney, although itself free of larvae may become heavily laden with concretions, and necrotic areas may appear, during infection (Hurst 1927).

Larval trematode infections may exert important lethal effects on the hosts. Wesenberg-Lund (1934) emphazized their importance in reducing the snail-population of certain Danish fresh-water areas. Similar effects were observed in Physa parkeri parasitized by larval trematodes (Cort, Olivier, and McMullen 1941); in this case death resulted from a destruction of the liver.

The laying down of fibrous capsules (Hurst 1927) and uncalcified fibromata (Faust 1920) around the

larvae has been recorded.

Other effects noted have been a reduction of the muscular tissues of the foot, hypertrophy of connective tissues, and a suppression of the number of amoebocytes (all Hurst 1927).

Effects of metacercariae on the hosts have not been seriously studied.

II. DESCRIPTION OF THE METACERCARIA.

(a). Materials and Methods.

Various species of slugs were collected from sites where it was known that brachylaemid infections occurred. The principal host species were Milax sowerbii Férussac and Agriolimax reticulatus Müller.

The parasites inhabited the renal tissues of the host. Before dissection, slugs were relaxed by placing in a 250 ml. conical flask containing about 25 ml. of water (Cragg and Vincent, 1952). Carbon dioxide was bubbled through, and the animals became relaxed within 15 minutes. The roof of the mantle was then removed, together with the underlying renal and cardiac tissues; the whole was transferred to a watch-glass containing either tap-water or "Helix Ringer" solution as described by Pantin (1946). On vigorous teasing, any larvae present floated free of the host tissues, and were capable of living in the medium for 4 - 5 days.

Most studies on metacercariae were carried out on living specimens in tap-water, and under normal cover-glass pressure. After some hours under these conditions, specimens became remarkably translucent, especially when viewed under conditions of "dark-ground illumination" as obtained by using a phase-contrast condenser aligned for a magnification of x 40, in

conjunction with a normal x10 objective. This method was particularly valuable for observing the ciliated ducts and so-called excretory system.

Stained preparations of metacercariae were best obtained by staining with Gower's Carmine and differentiating with chlorinated alcohol (Gower, 1939). Such preparations however were of little morphological value, as the animals tended to stain evenly throughout The genital glands, when present, were slightly more deeply-staining than were other structures.

Fixatives used for the parasites were Bouin's fluid, 70% alcohol, 2% formalin, formol alcohol, and F.A. 410. The best results were obtained with 2% formalin and formol alcohol.

In attempts to elucidate the reproductive system of the metacercariae, the following intra-vitam stains were employed: 0.5% aqueous solution of Trypan Blue; aqueous solution of Neutral Red Chloride; and a range of dilute aqueous solutions of Methylene Blue. Except Neutral Red Chloride, the stains failed to penetrate the animals unless the cuticle were pierced, which resulted in a rapid uniform staining; Neutral Red Chloride solution appeared to penetrate the cuticle, but also coloured the tissues uniformly, and was of no morphological value.

Unless otherwise stated, all drawings were made with the aid of a camera lucida, and all measurements taken by means of a micrometer eyepiece.

(b). The Morphology of the Metacercaria.

Studies by Krull (1935), Ulmer (1949) and Robinson (1949) showed that the cercariae of the Brachylaeminae possess caudal appendages, and that they develop into metacercariae in an intermediate host different from that which harbours the parent sporocyst and cercariae.

The presence of distinct stages in the development of brachylaemid metacercariae has been noted by a number of workers. Leidy (1850), although confusing two distinct species, described three stages in the development of metacercariae of Distoma vagans, including one characterized by a caudal appendage; Ulmer (1951), recorded three stages in the development of Postharmostomum helicis, recording the first as characteristically caudated.

In the present investigation, four distinct metacercarial forms of the brachylaemid are recognized, and designated caudal stage, feeding stage, corrugated stage, and smooth stage respectively. Their distinguishing features are shown in Table 2 and Figs. 1 - 5.

The measurements (mm.) given in Table 2 relate to larvae which were freshly removed from the host, and measured 'in vivo' under normal cover-glass pressure. Each vertical column is based on a random

TABLE 2

Measurements of Metacercariae (in mm.).

		caudal stage.	feeding stage	corrugated stage	smooth stage
Body.	Length	0.658 0.320-1.020	0.917 0.770-0.990	1.320 0.900-1.900	2.510 2.040-3.020
	Width (across ventral sucker)	0.260 0.200-0.375	0.535 0.340-0.900	0.681 0.380-0.880	1.020 0.860-1.280
Oral Sucker	Length	0.098 0.060-0.200	0.183 0.132-0.220	0.251 0.160-0.320	0.405 0.310-0.520
	Width	0.112 0.080-0.200	0.180 0.150-0.200	0.285 0.160-0.350	0.381 0.240-0.560
Ventral Sucker	Length	0.095 0.065-0.120	0.190 0.130-0.250	0.251 0.180-0.350	0.392 0.280-0.470
	Width	0.100 0.800-0.120	0.190 0.100-0.265	0.290 0.200-0.370	0.405 0.340-0.480

sample of 20 individuals; the single figures are the mean values for the samples, and the range of the sample in each case is recorded beneath the mean.

The youngest form was the caudal stage (Fig. 1), and these individuals appeared structurally indistinguishable from the preceding cercariae

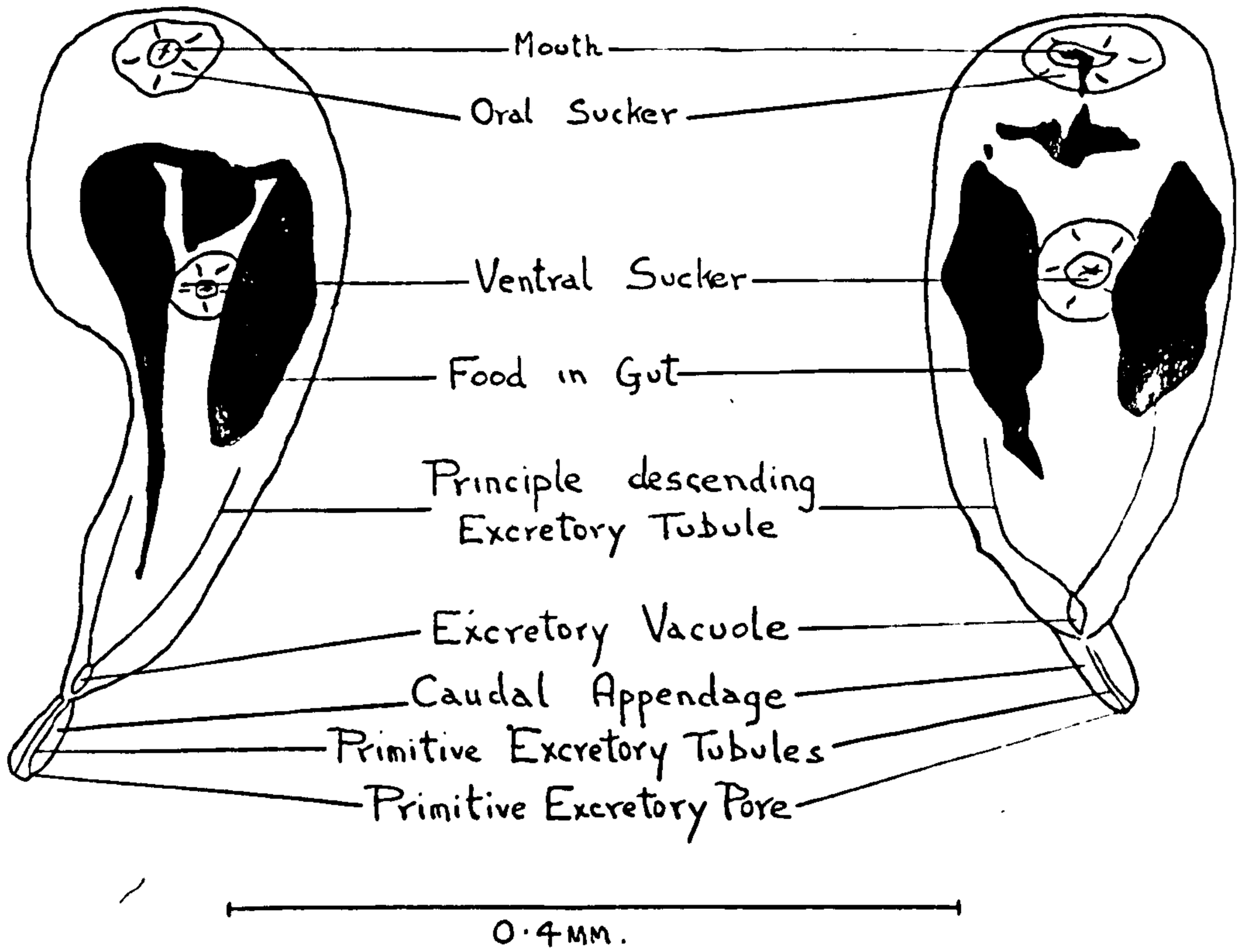


Fig. 1.

Caudal Stage Metacercaria.

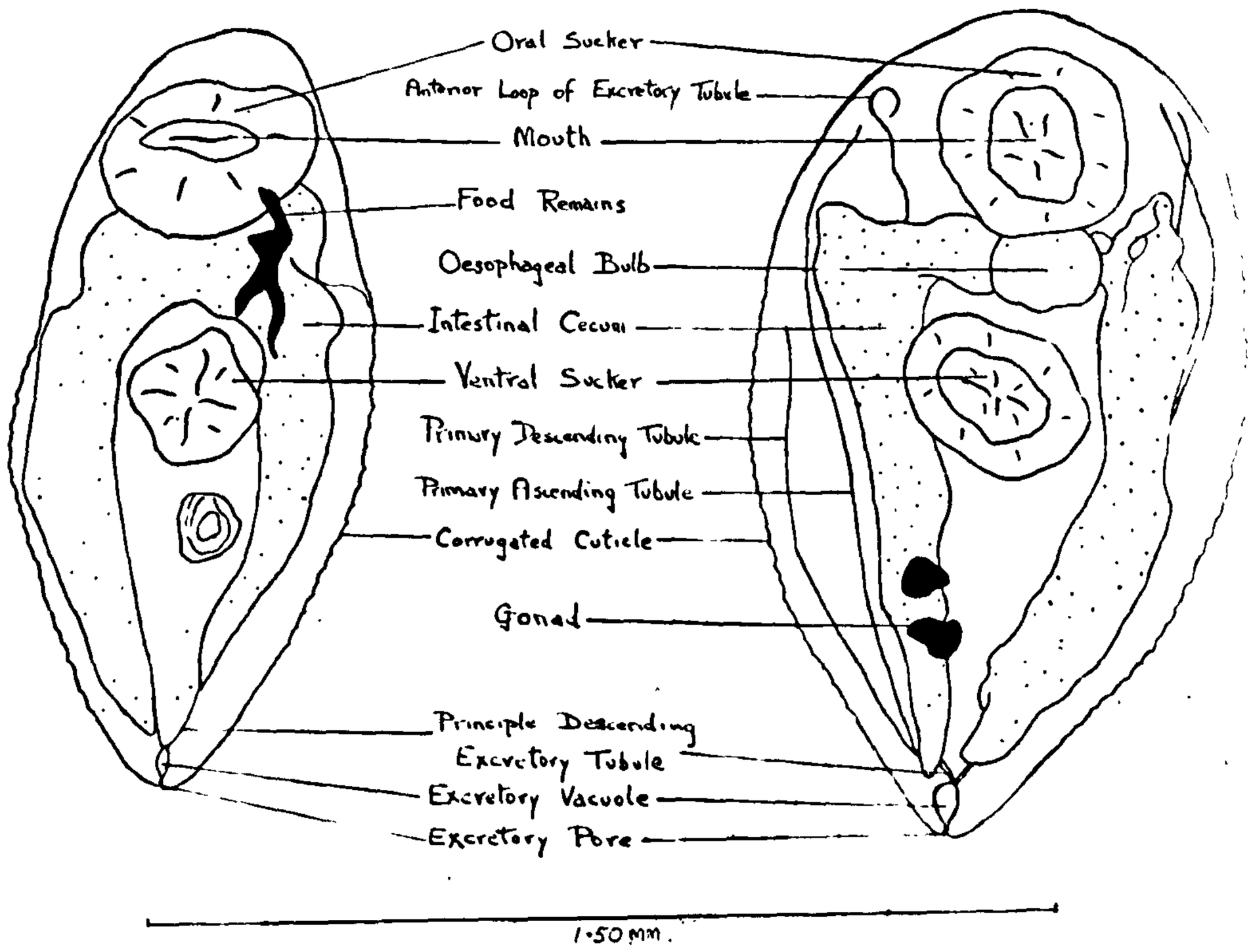


Fig. 3.

Corrugated Stage Metacercaria.

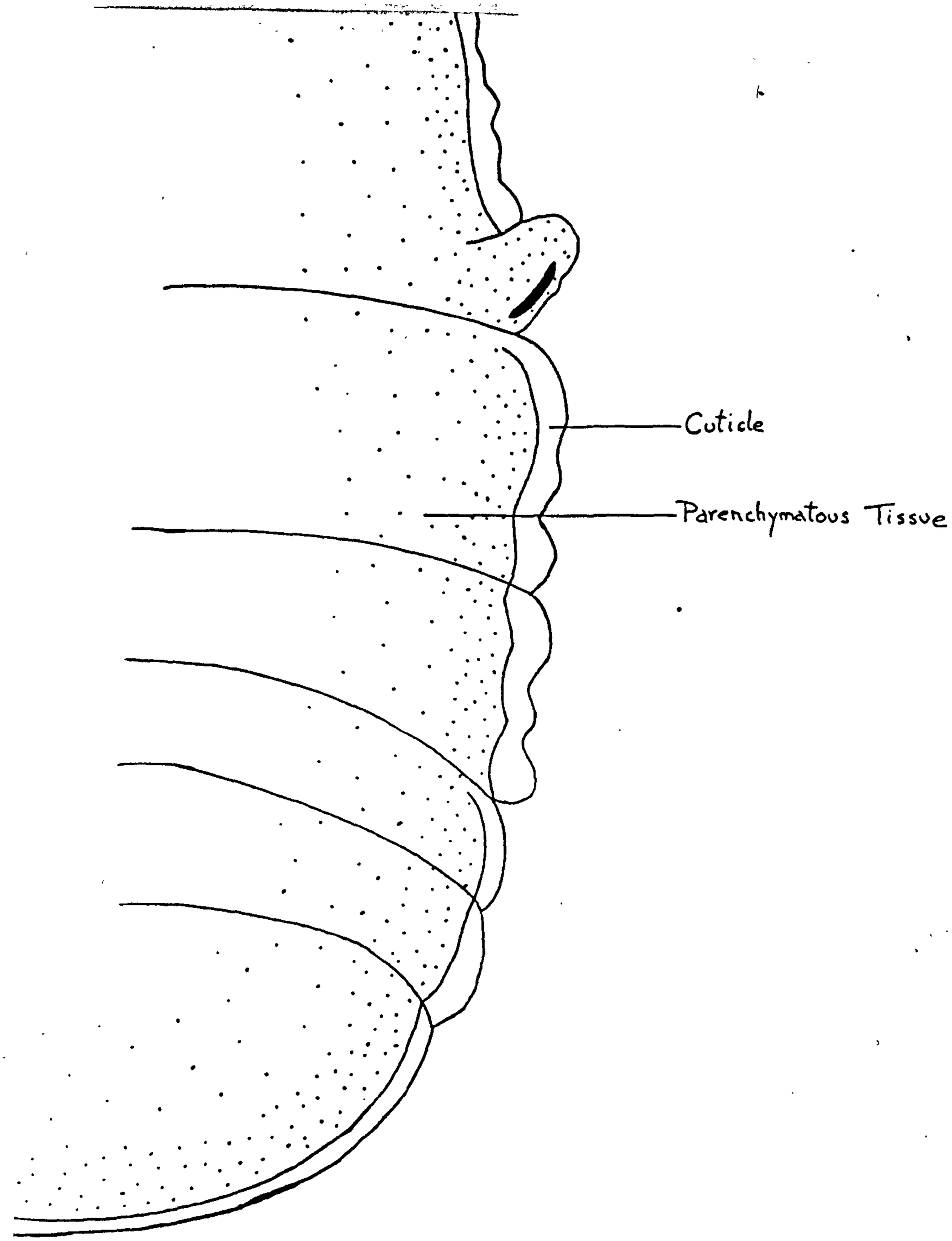


Fig. 4.

Cuticle of Corrugated Stage Metacercaria. x950.

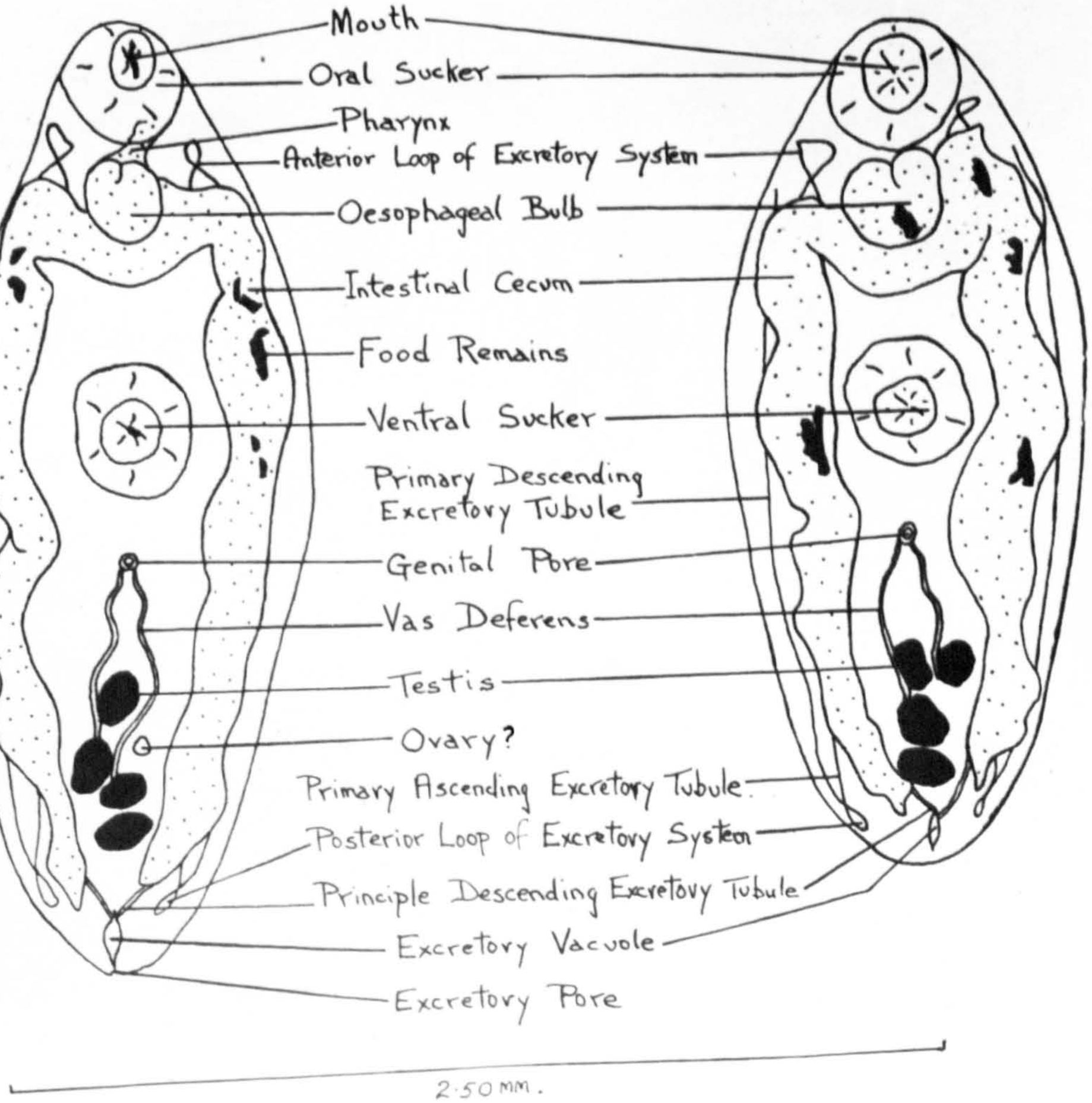


Fig. 5.

Smooth Stage Metacercaria.

(see section III (f)). The gut crura of the caudal stage larvae were often well-filled with food material, the nature of which will be discussed later. The principal descending excretory tubules opened into an excretory vacuole at the posterior end of the body, and were then continued throughout the length of the caudal appendage as a pair of primitive excretory tubules, each opening by a primitive excretory pore at the distal extremity of the appendage.

The caudal stage was succeeded by the feeding stage (Fig. 2). This was characterized chiefly by the presence of a densely packed, often refractory mass within the gut. The caudal appendage had now disappeared, and the excretory vacuole opened by a single pore at the posterior tip of the body.

The feeding stage was succeeded by the corrugated stage (Fig. 3), so designated on account of the cuticular corrugations. The gut was now quite empty except for a few scattered crystalline masses. The rudiments of the adult gonads occasionally appeared during this stage as darkly-staining bodies, but their appearance was usually delayed until the succeeding stage.

The cuticle of the corrugated stage larvae is shown in Fig. 4. The integument was highly irregular in the median parts of the body, the furrows extending completely across the surface of the animal. The

cuticle at the anterior of the body was only slightly furrowed, and the extreme posterior region often remained smooth. In the larger convolutions the cuticle often reached a thickness of 15μ or more.

The ultimate stage of metacercarial development was reached in the smooth stage (Fig. 5), in which the cuticular corrugations were lost, and "maturity" was attained. The living, infective metacercaria was grayish-white, ovoidal, and widest in the region of the ventral sucker. When motionless it became concave ventrally and convex dorsally. The cuticle was relatively thick, varying between (approximately) 5μ and 12μ, with a mean of about 9μ. It was smooth, devoid of spines or sensory papillae, and no cyst was present at any time. There was no jelly-like membrane such as occurs in some Leucochloridiinae and others. This membrane, the origin and function of which are unknown, and which is not to be regarded as a true cyst wall (Ulmer 1951), is conceivably replaced by the thick cuticle of the present species.

A circular oral sucker, subterminal and ventral in position, surrounded the mouth, which led into a short, thin-walled pharynx. The oesophagus was short, bulbous, and thick-walled. The gut bifurcated immediately into two crura which usually ran towards the anterior lateral corners of the body, and then turned and ran straightly, extending to the posterior limit of the body.

The ventral sucker was spherical, median, and very slightly to the anterior of the equatorial region.

Unlike many metacercariae, the reproductive system of this species is difficult to elucidate, and appears to be in an early stage of development. This is of interest in view of the comment of Dujardin (1845) that in larval brachylaemids in molluscs no genital organs were visible. It is possible of course that rudiments of such organs were in fact present, but were overlooked by that author.

Genital glands sometimes appeared in the posterior third of the body towards the end of the corrugated stage, but more usually did so during the smooth stage. They arose as a number of spherical, darkly-staining bodies, usually three or four in number but occasionally five. They all appeared similar to each other, even in section, and there was no/certain evidence of any differentiation into ovary and testes. The genital pore was circular, lying approximately one quarter of the distance between the ventral sucker and the posterior margin of the body. From the region of the gonads there arose two indistinct tubes, which ran parallel to each other to the genital pore; these were presumably the vasa deferentia. There were no signs of oviduct, ootype, Laurer's canal. vitelline system, or uterus such as have been recorded in other

larval brachylaemids. These female organs may develop only when the metacercaria enters the vertebrate host.

It is now generally accepted that the excretory systems of trematode larvae are of taxonomic importance, and that descriptions of these structures should be based on living larvae, and not exclusively on stained preparations.

Ciliated excretory ducts are known to occur in many Brachylaeminae. Sinitsin (1931) referred to the presence of such ducts as one of the "constant elements" of brachylaemid excretory systems. Ulmer (1951) however, pointed out that at least the genus Postharmostomum is diagnostically separated from the remainder of the subfamily Brachylaeminae by the absence of ciliated ducts. Ciliated ducts occur in the present species. The excretory system was difficult to observe, and has never been seen in its complete form at any one time. The following description resulted from a series of observations, and may conceivably be incomplete; it is unlikely, however, that any major parts have not been seen.

The course of the main ducts differed from that of other species, and is shown in Fig. 6. Careful scrutiny has failed to reveal any flame-cells, and these structures are considered to be absent in the species.

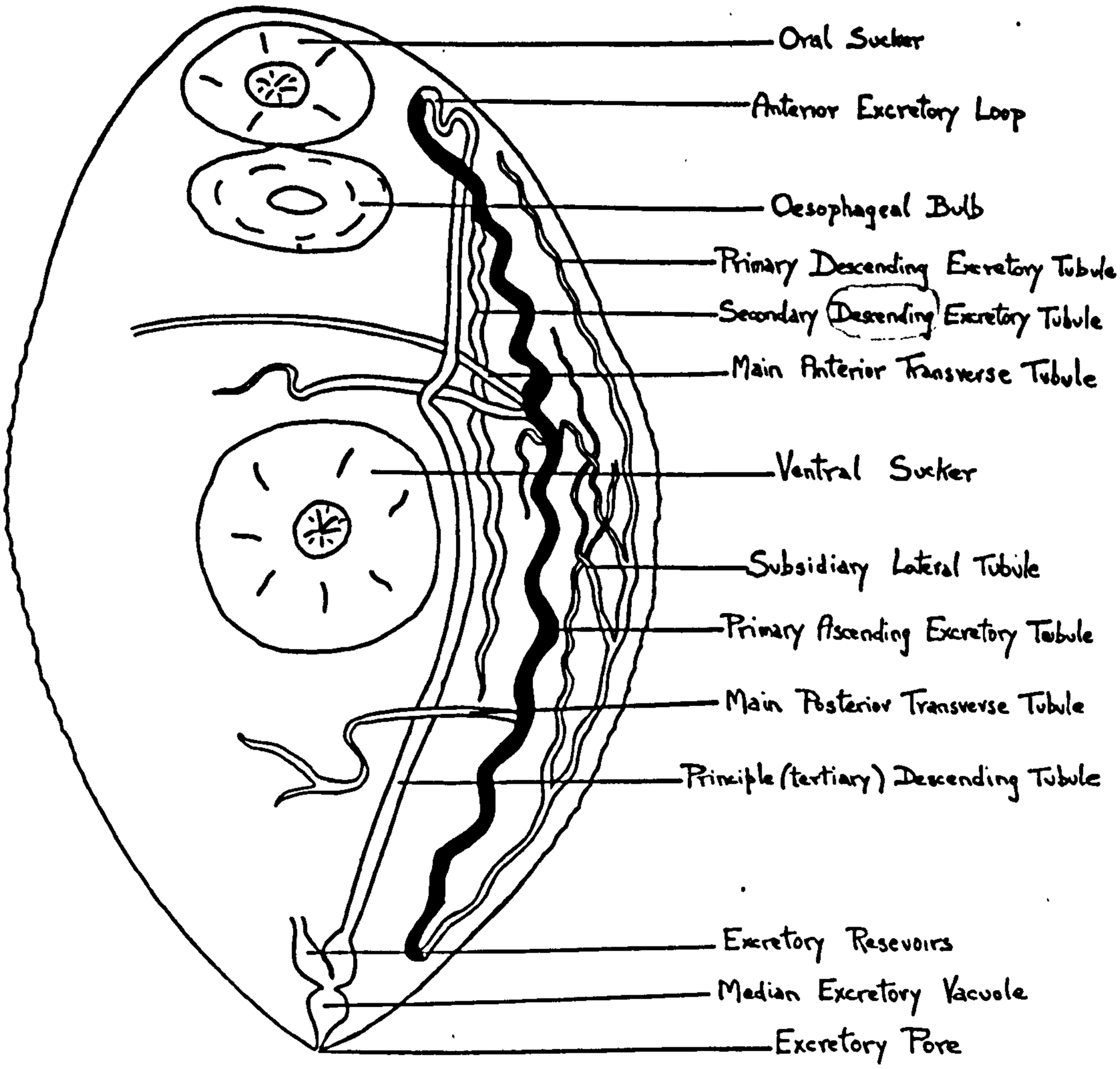


Fig. 6.

Excretory Canal System of Metacercaria.

Composite drawing; right-hand side only.

Ciliated tracts shown in solid black.

The distal opening of the canal was in the lateral region of the body, slightly posterior to the level of the oral sucker. From this point, the primary descending tubule ran to the posterior, following the line of the lateral surface of the body and receiving three subsidiary lateral tubules, two in the region of the ventral sucker and one at a point approximately half-way between the ventral sucker and the posterior extremity of the body. Posteriorly, the primary descending tubule turned back upon itself with or without a figure-of-eight lopp (posterior loop of the excretory system), and ran forward in a convoluted manner to the extreme anterior region. This primary ascending tubule was the only part of the system to be ciliated. The cilia were fairly long, and beat rapidly and continuously in a posterior-anterior direction. About the level of the oesophagus the ciliated duct received another convoluted , but unciliated duct, designated the secondary descending tubule. This ran parallel to the ciliated duct into the region just posterior to the ventral sucker.

Slightly anterior to the level of the ventral sucker two further subsidiary ducts arose from the ciliated duct. The posterior one ran transversely, finishing in a median position; the anterior one, designated the anterior transverse tubule, appeared

in some specimens to traverse the body completely and unite with the ciliated duct of the other side. Leidy (1850), Dujardin (1845), and Mehra(1936) have recorded complete cross-connections in brachylaemid excretory systems, although the former two authors are not noted for accuracy. Leidy in particular used the term "anastomose" very loosely. (see Discussion, p.141).

A further transverse tubule, the main posterior transverse tubule, arose from the ciliated duct just posterior to the ventral sucker.

The ciliated duct, on reaching the anterior of the animal, looped back upon itself (the anterior loop of the excretory system) and continued to the posterior extremity of the animal, usually with an indentation just anterior to the ventral sucker. This principal or tertiary descending tubule was enlarged in the region posterior to the ventral sucker, and opened into an excretory reservoir. The two reservoirs opened into a median excretory vacuole, which was thin-walled and situated between the tips of the intestinal crura; it discharged to the exterior by way of a single terminal pore.

The excretory vacuole was highly contractile. If the animal were placed in Helix Ringer solution (Pantin 1946) at room temperature, the vacuole discharged approximately five or six times per minute.

On removal from the host and placing in a suitable medium (saline solution or water), the parasites exhibited considerable activity, the younger stages being more active than the older ones. Fig. 7 illustrates the successive positions adopted by a feeding stage larva in Helix Ringer solution, drawn at ten-second intervals. Movement of this type in mature larvae indicated that no rigid cyst was present. Movement ceased after an hour or so, although sporadic movements, especially on mechanical stimulation, were observed up to five days after removal from the host.

(c). Location and Number of Parasites.

Metacercariae of all stages infected only the renal tissues of the host; none were encountered in any other organ. The caudal stage and feeding stage larvae were attached to the kidney lamellae by means of the ventral sucker, as shown in Plate 1, Fig. 8. The renal lamellae were distorted and drawn into the cavity of the sucker. The corrugated stage and smooth stage individuals lay freely in the cavity formed within the kidney as a result of the action of the feeding stage larvae (see Section VI (b)).

The number of parasites supported by a host decreased as each metacercarial stage succeeded the preceding one, although great variations occurred in

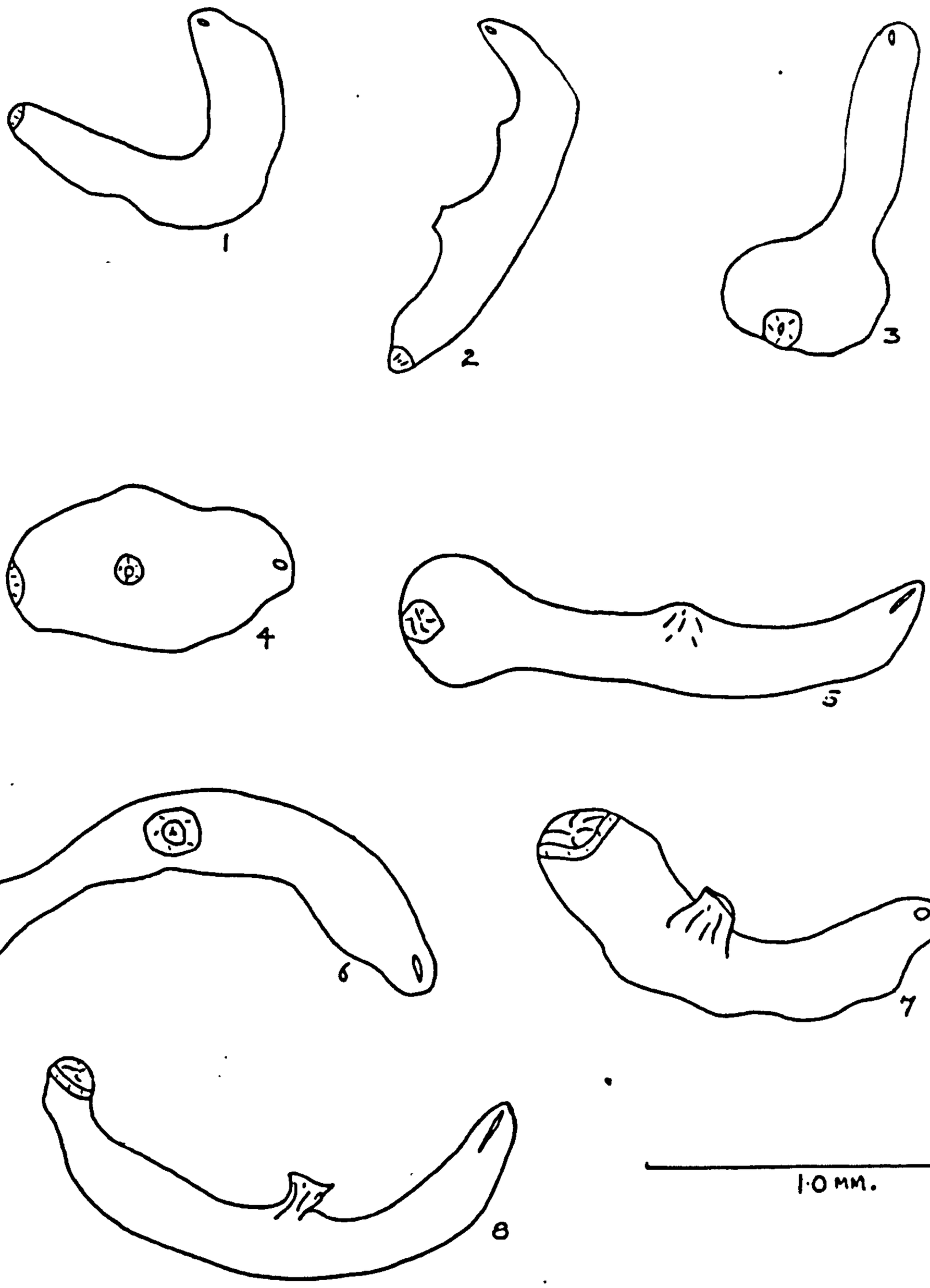


Fig. 7.

Shapes adopted by Metacercaria removed from Host and placed in saline solution.

(Free-hand sketches at ten-second intervals).

PLATE 1.

Fig. 8.

Metacercaria attached to Kidney Lamella by Ventral Sucker. x50.

some individuals. Caudal stage larvae usually numbered over 70 per host, and a number of individuals were found to harbour over 100 larvae. The average number of feeding stage larvae was about 25 per host, of corrugated stage about 10 per host, and of smooth stage about 6 per host. No slug was found harbouring more than 20 mature metacercariae. The significance of this progressive decrease in numbers will be considered in the general discussion.

(d). Parasite longevity and Host Immunity.

The data on the longevity of the larval stages is deduced from the field evidence to be presented in Section III (c). The caudal stage individuals had a life of approximately one month, and the succeeding feeding stage forms of 6 - 8 weeks. The corrugated stage lasted approximately a month, although occasionally as long as 2 - 3 months, and the smooth stage larvae could survive for at least four months including the period of overwintering. The total life-span of this brachylaemid metacercaria was thus possibly as long as eight months, although maturity could be reached after four months.

The presence within a single kidney of larvae of different developmental stages indicated that no effective immunity was developed by a host following

an initial infection. This was observed in both M. sowerbii and A. reticulatus.

No slug of a weight of less than 150 mg. was found to carry an infection, even in populations where up to 70% of the larger individuals were parasitized. Only rarely were slugs of a weight of 150 - 200 mg. found to be parasitized, but above 200 mg. the animals were infected regardless of age or size. This may indicate the presence of a natural immunity among the younger individuals, or a difference in the feeding habits of such forms.

The infection-free young individuals occurred in both species of hosts.

(e). Growth of Metacercariae.

In the absence of "controlled" (i.e. laboratory) infections, the rate and mode of growth of metacercariae within the host was determined as follows.

The data for each stage have been based on a random sample of 20 individuals. In determining the size of individuals, account was taken of changes in shape by the multiplication of the length and breadth (across the ventral sucker) of the animal, the resultant multiplicand being termed the "size-factor." (Table 3). Alterations in the thickness were known from observation to be negligible, and were ignored.

The points were plotted as a logarithmic graph to reduce the scatter of individual points and to produce a more easily interpreted line than a curve. (Fig. 9).

TABLE 3.

Growth Rate of Metacercariae.

Developmental stage.	Mean "size-factor"	Log. Mean "size-factor"
Caudal	0.1270	$\bar{1}$.1038
Feeding	0.4060	$\bar{1}$.6085
Corrugated	0.9410	$\bar{1}$.9736
Smooth	2.5240	0.4021

Fig. 9 shows the graph of the means to be a straight line, indicating a steady development from caudal- to smooth stage individuals. There was no sharply defined cross-over point between consequent stages.

(f). Decaudation.

From observations of "wild" individuals, decaudation of caudal stage metacercariae is considered to be a gradual process resembling that described for other brachylaemids by Leidy (1850) and Ulmer (1951).

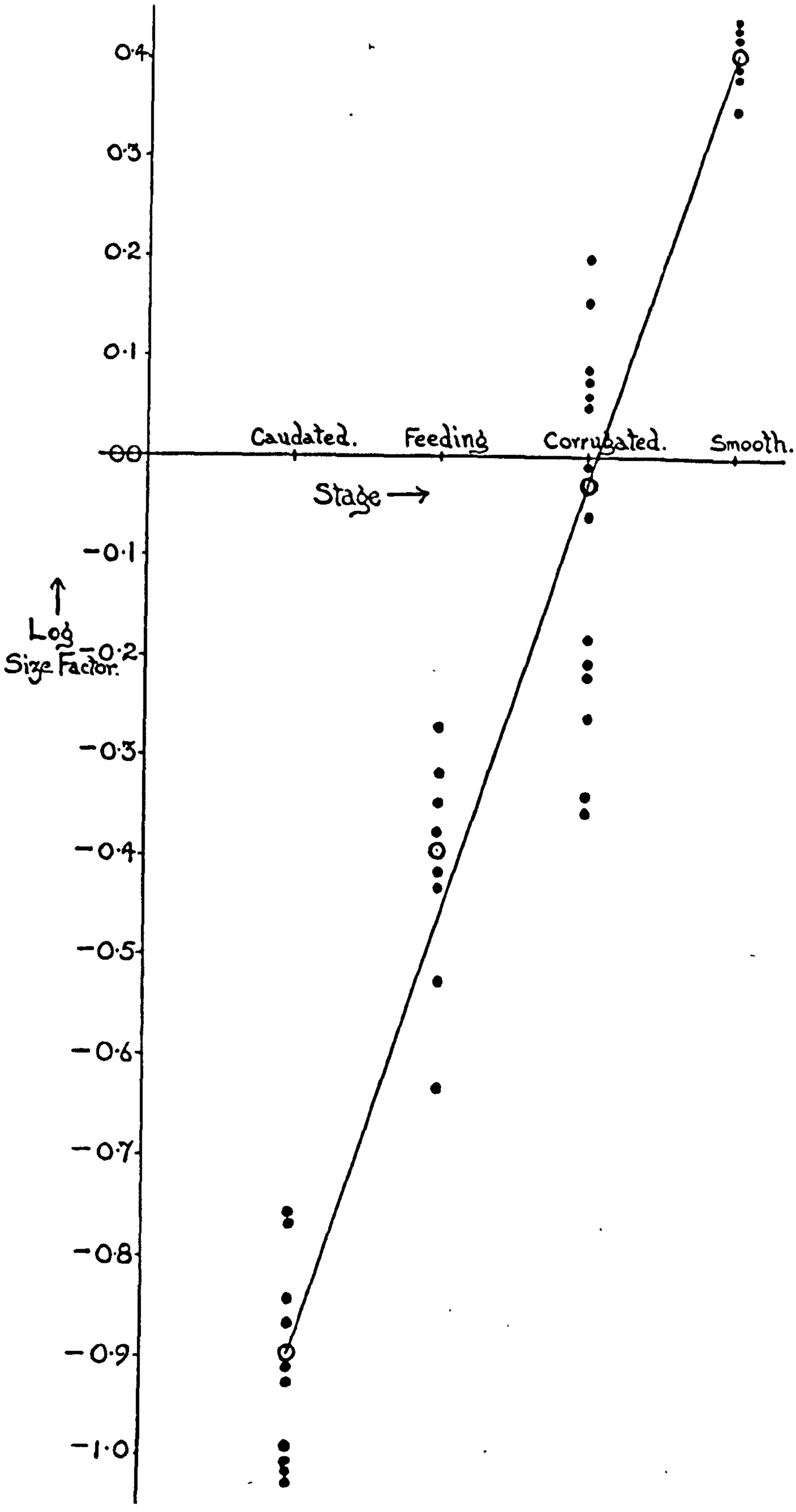
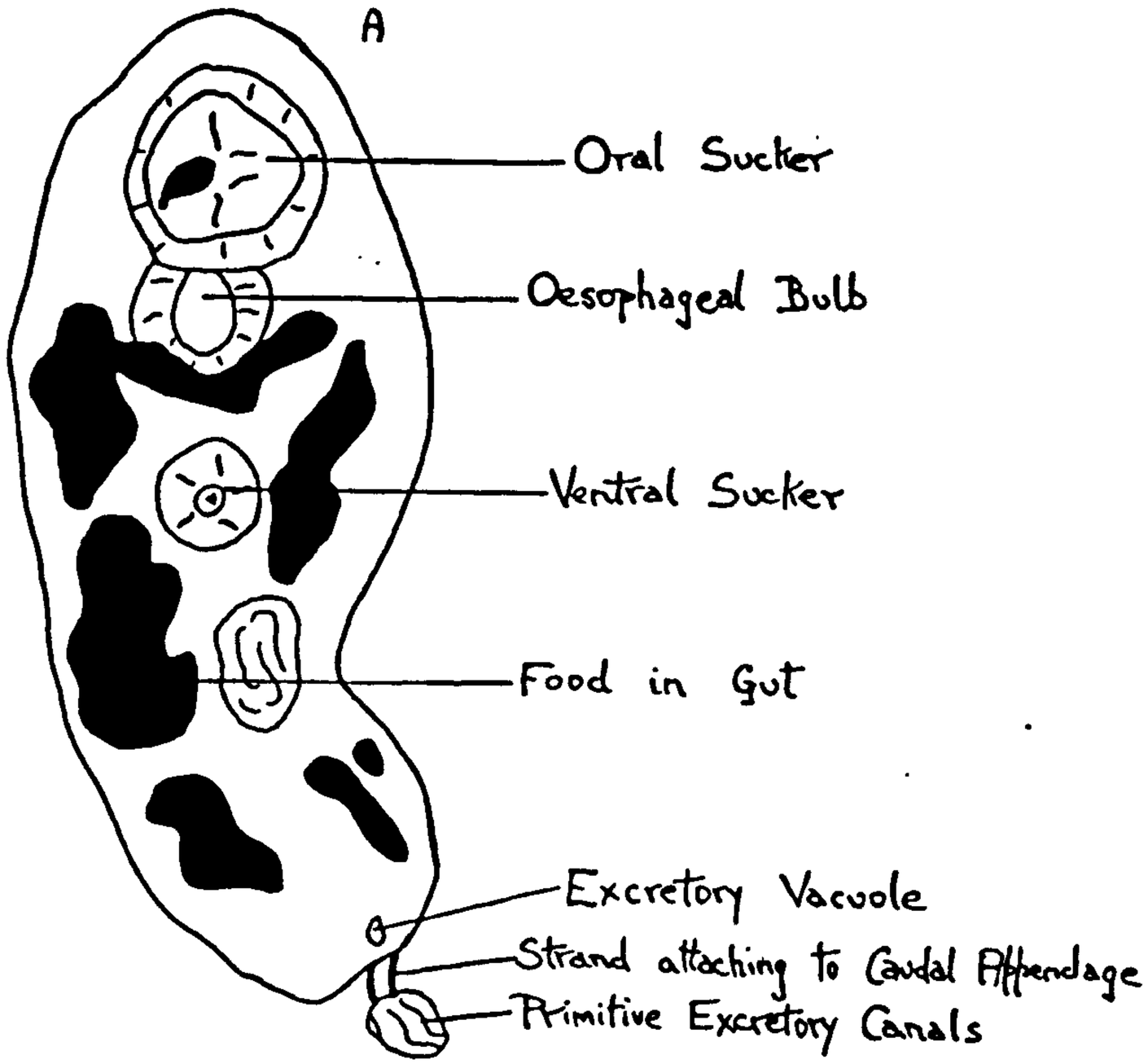


Fig. 9.
Development-
Rate of
Metacercariae.



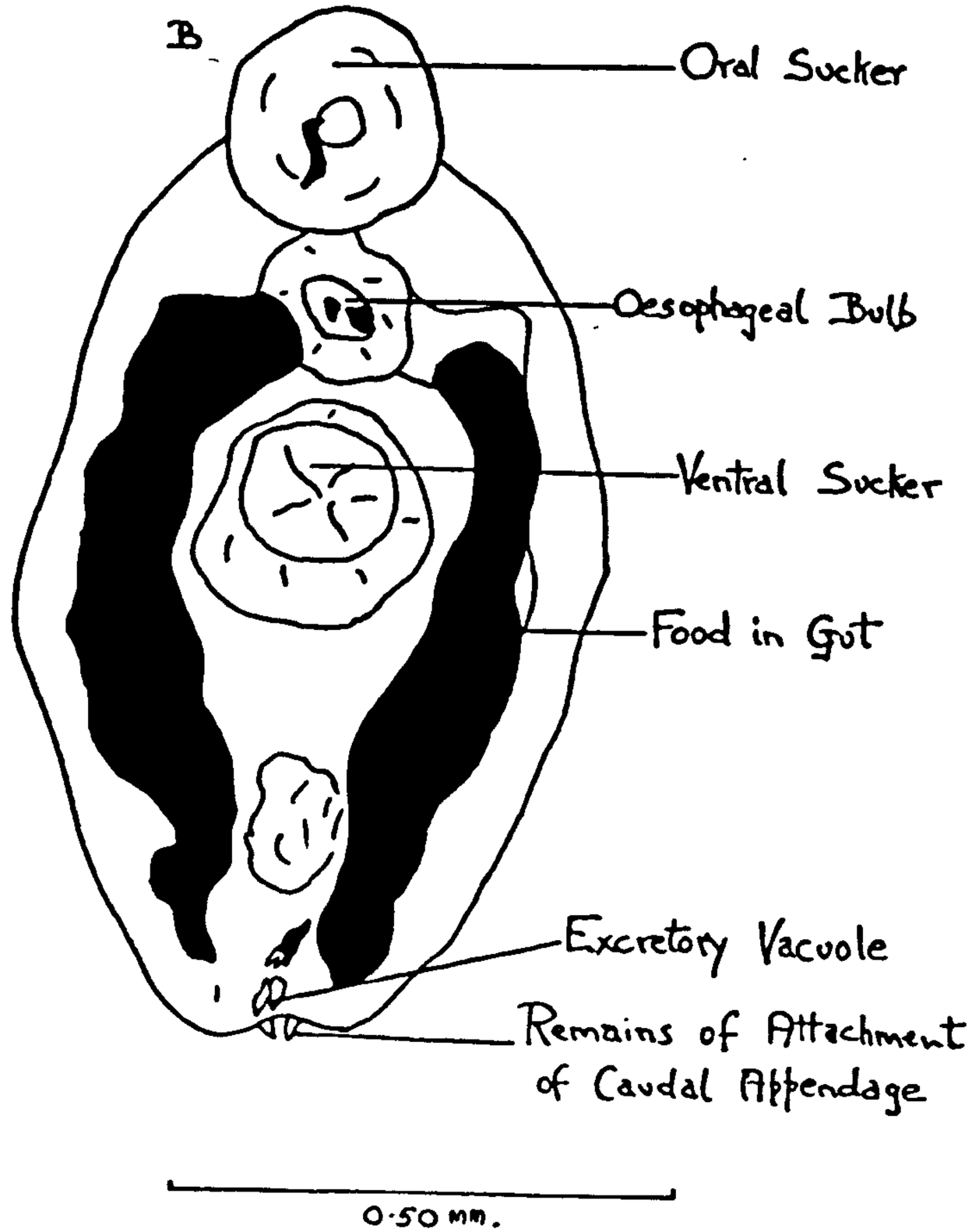


Fig. 10

Decaudation of Metacercariae

Towards the end of the caudal stage the primitive excretory canals of the appendage (Fig. 1) became non-functional, and a single median excretory pore opened at the tip of the main body. This had the effect of dividing the dorsally attached caudal appendage into two laterally situated stems (Fig. 10A). The two strands of tissue which attached the appendage to the body ultimately broke and completed the process of dehiscence. It is unknown whether or not they broke simultaneously.

Feeding stage larvae have been seen bearing two fragments of tissue at the posterior extremity (Fig. 10B). These were conceivably the shrivelling remains of the strands formerly attaching the caudal appendage to the body.

III. OBSERVATIONS ON INFECTION.

(a). Materials and Methods.

Slugs were collected in half-hour periods, usually about 19.00 - 22.00 hrs. G.M.T., with the aid of an electric torch. Slug traps were not used. A few collections were made during daylight, and no specific differences were noted in the contents of such collections and those taken after nightfall. In determining the incidence of infection in a population, samples of 20 or more were considered significant.

Animals employed in feeding experiments to determine a suitable definitive host were laboratory bred mice, chickens of various ages from 4 days to 12 weeks, guinea-pigs, and "wild" hedgehogs.

Metacercariae were removed from slugs and placed in a few drops of water. Approximately 2 ml. of the charged solution were orally injected into the vertebrate by a glass pipette. The vertebrate was fed normally about 30 minutes before the injection. Guinea-pigs were lightly anaesthetized with ether to prevent breakage of the pipette, which in their case comprised a piece of wide-bore capillary tubing fused on to a normal pipette stem.

In other cases a small amount of "Grower's Mash" was moistened with water containing larvae, and fed to vertebrates which had been starved for 24 hours.

Sometimes larvae were added to the drinking water of animals previously deprived of water for 24 hours.

Attempts were made to feed either whole slugs or their mantle region to chickens. The "cloacal drop" method (Allison 1943), in which larvae were placed on the cloacal lips and thus subjected to rhythmical muscular contractions which drew them up into the rectum, was also employed.

Oral injections were unnecessary with hedgehogs, as these animals devoured whole slugs avidly. They were simply allowed to eat anaesthetized slugs, some of which were known to harbour mature larvae.

The faeces of all vertebrates employed were examined periodically for trematode eggs. The method employed was due to Taylor (personal communication) and is recorded in Appendix II. The method of concentrating eggs in faeces described by Gates (1921) was also used, substituting a saturated solution of zinc sulphate for the calcium chloride solution described.

Field-mice and voles were trapped, and their guts examined for parasites. Acorns, nuts, dried peas and crocus corms were used as baits.

(b). Seasonal Fluctuations in Incidence of Infection.

The highest incidence of infection was found in the garden of 25, North Bailey, Durham, and showed definite seasonal fluctuations which are recorded in Tables 4 and 5 and Fig. 11.

The data are based on collections of slugs from this garden between September 1951 and February 1953. The figures for after this period showed peculiarities and are treated separately in Section V (p.75).

The monthly totals in Tables 4 and 5 do not include slugs of less than 150 mgs. in weight owing to the inability of such individuals to support an infection.

Table 4 and Fig. 11 show the seasonal incidence of infection in A. reticulatus, and Table 5 summarizes the data for M. sowerbii.

Metacercarial infection was in the early spring, about March or April. Fig. 11 shows that after this spring infection, the incidence rose rapidly, reaching the 90 - 100% mark in June and July. During August there was a sharp drop resulting from the death of a large number of parasitized individuals and their replacement by younger and infection-free individuals. The level of infection during autumn and winter showed a slow but steady decline, the minimum values occurring about February, just prior to the spring increase.

TABLE 4

Seasonal Incidence of Infection in A. reticulatus.

Month.		Total No. of hosts examined.	% age Infection.
Sept.	1951	26	38
Oct.		9	22
Nov.		47	25
Dec.		30	13
Jan.	1952	30	6
Feb.		22	5
March.		27	8
April.		39	45
May.		31	42
June.		62	95
July.		50	97
Aug.		30	50
Sept.		23	48
Oct.		32	40
Nov.		27	33
Dec.		32	25
Jan.	1953	32	37
Feb.		44	27

TABLE 5

Seasonal Incidence of Infection in M. sowerbii.

Month		Total No. of hosts examined.	% age infection.
Sept.	1951	16	63
Oct.		3	66
Nov.		7	71
Dec.		00	-
Jan.	1952	20	50
Feb.		21	57
March.		1	(100)
April.		11	27
May.		35	60
June.		00	-
July.		00	-
Aug.		00	-
Sept.		00	-
Oct.		29	55
Nov.		21	62
Dec.		7	52
Jan.	1953	9	22
Feb.		10	00

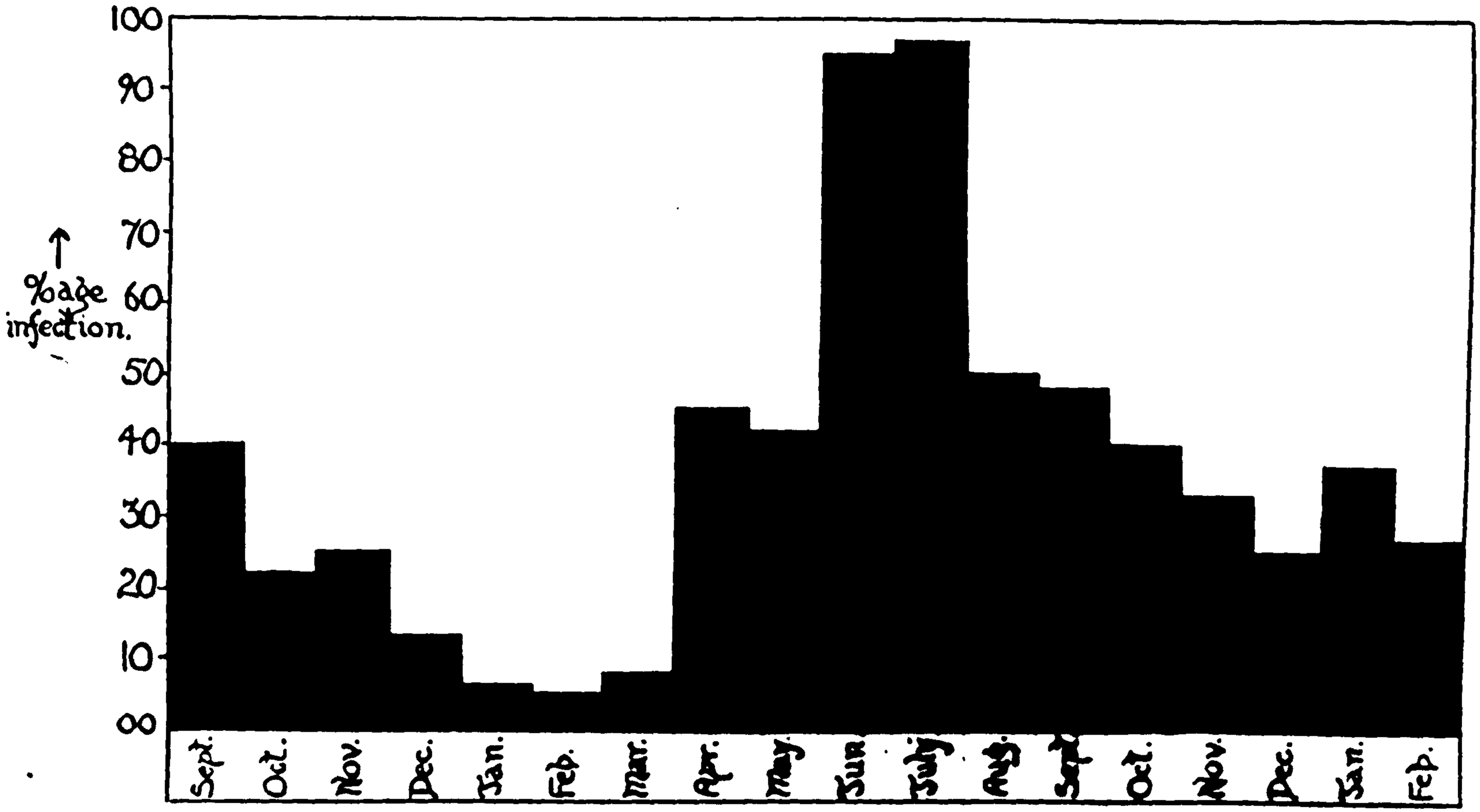


Fig. 11

A.reticulatus: Seasonal Incidence of Infection

The slight rise in the incidence of infection during October and November 1951, and January 1953 resulted from a secondary autumnal infection of slugs by metacercariae developed from adult parasites during the late summer; this is referred to later.

Owing to the comparative scarcity of M. sowerbii in the area, complete records for the species are not available. The data obtained are given in Table 5. No violent fluctuations in the level of infection such as occurred in A. reticulatus were observed. Further, infection was consistently at a higher level than in Agriolimax. This point will be enlarged upon later (p.73). From Table 5 it appears that the incidence of infection in the M. sowerbii reached a peak in the late summer-early autumn, and that infection fell during winter and early spring. While both species showed the same general pattern, the fluctuations in M. sowerbii were obviously smaller in magnitude; this was largely because of a greater tolerance of the effects of the parasitism (see Section VI (e)).

(c). Seasonal Fluctuations in Incidence of Infection of Developmental Stages.

As expected, infections at different seasons were composed of different proportions of the developmental stages. Table 6 and Fig. 12 show the seasonal variations

in the occurrence of the four different growth stages, the figures being derived from the collections referred to in Fig. 11. Hosts carrying more than one stage of parasite simultaneously were included, but were found to be rare.

The spring rise in infection was due to the appearance of a large number of young caudal stage metacercariae. (Fig. 12). These forms reached a peak in numbers in late March, dropped slightly during April, and then rose to a maximum during May. There was then a rapid decline in numbers, a slight revival during July, followed by the extinction of the stage in August.

The same general trends are evident, after the appropriate lapse of time (p.43), in the histograms representing the succeeding stages. The previous observation that the corrugated stage may be longer-lived than the others is supported by the fact that it is absent from the fauna for a shorter period of the year than are the others.

Fig. 12 shows a secondary infection by caudal stage larvae during November - December. This was followed, after an interval of a month, by a subsidiary peak of feeding stage individuals. That subsequent rises did not occur in the corrugated and smooth stage histograms was due to the failure of these individuals to progress further, and to their stagnation as

TABLE 6.

Seasonal Variation in Growth Stages of the Parasite.

Month.	No. of Hosts Examined.	% age of stages among infected Individuals.			
		CAUDAL	FEEDING	CORRUGATED	SMOOTH
Sept. 1951	26	00	00	29	72
Oct.	9	00	00	33	67
Nov.	47	00	00	50	50
Dec.	30	9	00	45	45
Jan. 1952	30	00	5	30	70
Feb.	22	00	00	21	73
March.	27	75	10	15	00
April.	39	50	50	00	00
May.	31	79	21	00	00
June.	62	4	69	27	00
July.	50	9	40	51	00
Aug.	30	00	4	32	64
Sept.	23	00	5	45	50
Oct.	32	00	00	50	50
Nov.	27	4	1	25	70
Dec.	32	1	5	20	75
Jan. 1953	32	00	00	20	80
Feb.	44	00	00	20	80

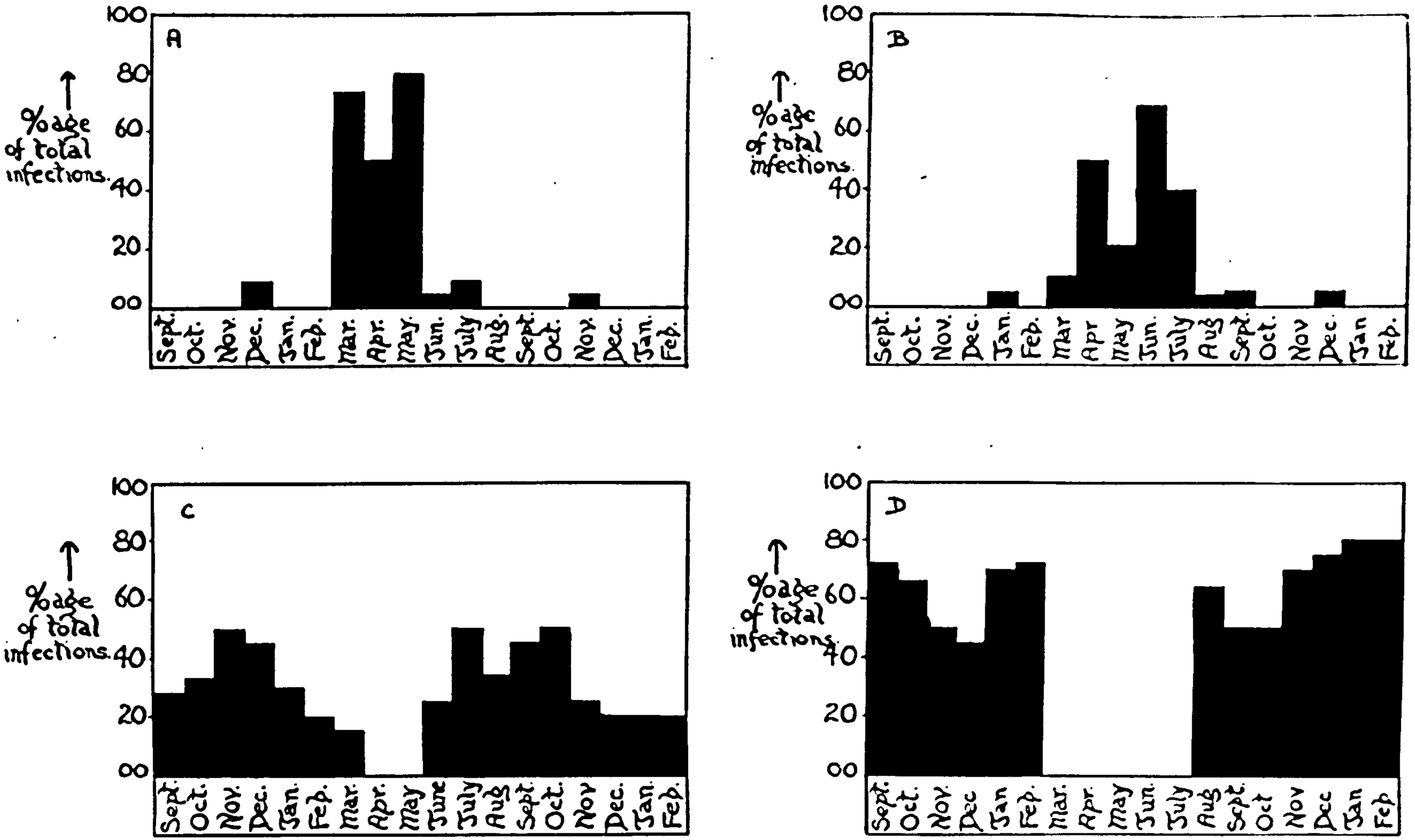


Fig. 12

Seasonal Variations in Growth Stages of Parasite .

A: Caudal Stage
B: Feeding Stage
C: Corrugated Stage
D: Smooth Stage

atypical, feeding stage larvae. The material in the gut crura remained as a loosely-packed, greenish, non-crystalline mass, and the cuticle showed only occasional corrugations. They increased very slightly in size, remained like this throughout the winter, and finally died out during the following spring with the true corrugated-and smooth stages.

It is possible that the unfavourable climatic conditions acting indirectly upon the larvae through the host caused these secondary autumnal infections to die out without reaching maturity.

(d). A possible Life - history.

The ecological studies described above may enable us to postulate the main trends of the parasite's life-history.

All known Brachylaeminae utilize either birds or mammals as the primary host. Considering the limited distribution of this species, it is thought unlikely that a bird is involved, even taking into consideration such phenomena as the "territory" of many birds. It is more probable that a mammal is the vertebrate host, and the terrain of the infected locality appears suitable for such animals as mice, voles, and hedgehogs. Furthermore, hedgehogs have been observed within a

reasonable proximity of no. 25 North Bailey, and these animals, together with voles and shrews, have been listed by previous authors (Stiles and Stanley 1932; Dawes 1946) as definitive hosts for brachylaemid species, the former authors listing both Arion sp. and Agriolimax sp. as the intermediate hosts.

Approximately 15 "break-back" traps were set nightly for a period of six weeks during the spring of 1953 in 25 North Bailey, others being set intermittently during the same period throughout the area of collection shown in Fig. 13. No mammals were caught (see Section V).

It is suggested that the vertebrate host is a small mammal, possibly the hedgehog, which eats slugs readily. The vertebrate is infected during late autumn - early winter, the parasites coming to maturity during the period of hibernation or decreased activity. With the resumption of full activity in spring, brachylaemid eggs are passed with the faeces and ingested by browsing slugs. Parthenitae result, and metacercariae appear about March - April.

Those appearing first will mature in late summer, and the vertebrate may thus acquire an infection which allows viable eggs to be passed before the onset of winter inactivity. A few slugs therefore acquire a larval infection in the late autumn, so accounting for the secondary metacercarial infection of November to December. (Fig. 12).

Most adult flukes, however, overwinter in the vertebrate, passing viable eggs during the succeeding spring.

A possible correlation between the known invertebrate host and the proposed vertebrate host lies in the nocturnal activity of each. The nature and time of activity of slugs was investigated, and the results are recorded in Appendix I. It is felt that although slugs may both ingest trematode eggs and be ingested themselves by vertebrates when lying quiescent during the day, both phenomena are more likely to occur during movement in the open at night.

Thus if both vertebrate and invertebrate hosts are nocturnal animals, the effect may well be to raise the chances of infection. If a bird were involved, many hours of possible dessication may occur between the passing of the parasite's eggs and their ingestion by a slug. Conversely, nocturnal vertebrates are more likely to encounter trematode-infected molluscs.

(e). Studies on possible Vertebrate hosts.

(i). Field Studies.

It was hoped to trap, in the brachylaemid-infested area, small mammals carrying adult brachylaemids. Between October 1952 and April 1953, spring-back traps were set in some 700 attempts in the area shown in Fig. 13, mainly at 25 North Bailey. No mammals were caught, although holes and runs were observed. This may have indicated a dying-out of the population during the winter. Since it was followed in the spring of 1953 by a great reduction in the brachylaemid population of the same area, the possible correlation of these two phenomena is discussed in Section V.

During the winter and spring of 1952/53, some 50 specimens of Apodemus sylvaticus L., Microtus agrestis L., and Clethrionomys Tilesius sp. were trapped at a number of Durham localities other than North Bailey. All were examined for trematode infections, and all proved to be free from such parasites.

Specimens of fox, badger, and occasional birds from the Durham area were also examined. None showed any trematode infection.

(ii). Laboratory studies.

Mice, guinea-pigs, and chickens were given oral injections of 6 - 50 mature metacercariae. Hedgehogs were fed on slugs of both host species from 25 North Bailey, one animal consuming 581 potential hosts (79 *M. sowerbii* and 502 *A. reticulatus*) over a five-week period; of these, it is estimated that approximately 140 were infested with metacercariae. Slugs were anaesthetized before being supplied to the vertebrate in order to immobilize them and enable ingestion to be watched.

Metacercariae were removed from slugs which had died either naturally or as a result of the parasitism (see Section VI (e)). Up to 48 hours after the death of the hosts, the parasites remained alive and were indistinguishable from larvae removed from living hosts. These larvae from dead slugs were also injected into the vertebrates.

The faeces of all animals exposed to infection were regularly examined for trematode eggs (Appendix II). In no case were any brachylaemid eggs found.

All animals exposed to infection were ultimately killed, and the gut tissues and contents examined under a binocular microscope; this was done at various intervals from one week to three months after exposure to infection. The following organs of chickens were

thoroughly examined: Crop, small intestine, colon, caecae, rectum, bursa fabricii, liver, kidney, and spleen. In mammals the organs examined were: Oesophagus, stomach, duodenum, ileum, colon, rectum, liver, kidney, and lungs.

No trematodes were seen in any animal, and it is concluded that these species are refractory to the parasite. The hedgehogs had heavy nematode infections (Capillaria aerophilla and Crenosoma sp.), and these may conceivably have led to an immunity to further parasitism.

Laboratory-bred hedgehogs could not be obtained.

(f). The Sporocyst Generation.

Before the present investigation was started, a single trematode sporocyst was found in a specimen of Arion ater L. from 25 North Bailey, Durham (Cragg and Vincent, unpublished). It was a large, branched structure, ramifying over the external surface of the digestive gland of the slug. Each branch was enlarged terminally into a sac-like structure containing approximately 10 cercariae. Although possibly unrelated to the metacercariae

forming the subject of this thesis, it appears likely that it was, in fact, the forerunner of these metacercariae, because the cercariae which it contained were structurally indistinguishable from the caudal stage metacercariae described on p.27.

Appearing in October, 1949, the sporocyst appeared to be of the secondary (autumnal) infection.

During the spring of 1953, a collection of 157 specimens of Arion ater from 25 North Bailey were dissected and examined for sporocyst infections. No infections were recorded, but this was not surprising in view of the generally low recorded incidence of sporocyst infections (1.1% of the host-population for Postharmostomum helicis, Ulmer 1951), and further in view of the subsequent decrease in the incidence of metacercarial infection in the area (Section V).

No host harbouring metacercariae was found to harbour sporocysts or cercariae. Thus it appears probable that, as in previously described brachylaemid life-histories, two intermediate hosts are involved, even if they are of the same species.

IV. THE GEOGRAPHICAL DISTRIBUTION OF THE PARASITISED SLUGS.

(a). The Area of Collection.

Table 7 gives a complete tabulation of all slugs from all sources examined for parasites between September 1951 and December 1953.

TABLE 7.

Summary of Molluscs examined: Sept. 1951 - Dec. 1953.

Host Species.	Number examined	Number infected with Metacercariae.	Other stages.
Agriolimax reticulatus.	1843	363	0
Agriolimax agrestis?	3	1	0
Milax sowerbii.	598	198	0
Milax gracilis.	2	0	0
Arion ater.	239	1	0
Arion circumscriptus.	23	0	0
Arion subfuscus.	8	0	0
Arion hortensis.	117	0	0
Limax maximus.	23	0	0
TOTALS.	2856	563	0

Arion ater L. and Arion rufus L. are recognized as distinct species, but no distinction has been made in the present work, both species being referred to collectively as Arion ater.

Of the 2856 slugs collected, about 90% were obtained from the bank of the River Wear between Elvet Bridge and Framwellgate Bridge, Durham. (Fig. 13). Some degree of infestation was found at all points of the stretch, the highest incidence being at 25 North Bailey. Of all slugs collected, approximately 62% were obtained from this point.

Smaller collections were made in the following localities: University Science Laboratories, Durham; Little High Wood, Durham; Houghall Wood, Durham; Bede College, Durham; St. Mary's College, Durham; Hallgarth Street, Durham; Gilesgate Moor, Durham (two localities); Shincliffe, Co. Durham; Darlington, Co. Durham; Allenheads, Wearhead, Co. Durham; Littleover, Derbyshire; South Hinksey, Oxford; and Weybridge, Surrey. Except for a few cases at Shincliffe and Bede College, and a single case at Hallgarth Street, Durham, no brachylaemids were found in the above localities.

The ecology and distribution of slugs, with particular reference to brachylaemid parasitization, was studied during 1952/3 along the bank of the River Wear shown in Fig. 13. The total length of territory examined was approximately ¾ mile, and collections were made at the nine stations numbered on the map.

Stations 1, 2, 3, and 9, were in open tree- or shrub-covered land with public access; stations 4, 5, 6, 7, and 8 were in private gardens, 6 and 8 being relatively uncultivated. Stations 5 and 7 possessed herbaceous borders, the others being kitchen gardens. Human

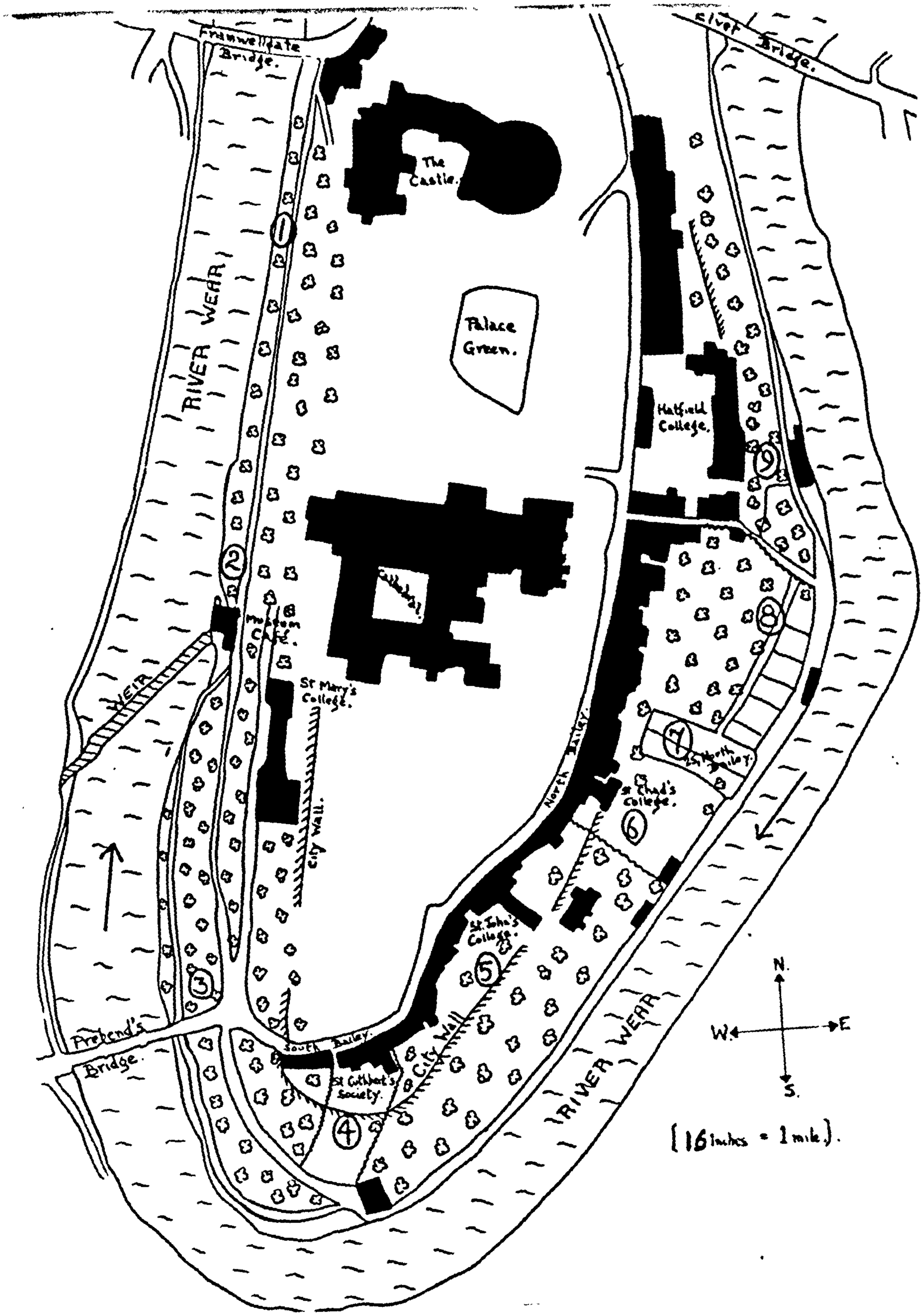


Fig. 13.
Area of Collection.

habitations in the area are shown in Fig. 13 in solid black; the corrugated lines running between the Bailey and the towpath represent the only walls traversing the area in an unbroken state and which might have divided it into a number of isolated blocks. That they failed to do so will be evident later.

Stations 1, 2, and 3, were all open to public access, but station 2 alone was further in close proximity to a café, the others being well removed from human habitations.

(b). Distribution of parasitism.

Table 8 shows the winter slug population of the region. In order that the samples from all stations might be comparable, all slugs were collected simultaneously by a team of collectors, two working at each station. Table 8 records the numbers of each slug species collected in a 45-minute period between 20.30 hrs. and 21.30 hrs G.M.T. on 27th November, 1952. The weather was fairly dry, with no wind, and a ground temperature of 5°C.

Of a total of 285 slugs collected, A. reticulatus comprised about half, while M. sowerbii accounted for about a tenth. The two host species were generally distributed throughout the area, but Arion sp. appeared to be mainly around the walls of stations 5 - 9.

TABLE 8

Slug Population Survey: 27th Nov. 1952.

Station.	Number present in 45-minute sample.				
	Arion ater.	Arion hortensis	Arion circumscriptus	Milax sowerbii	Agriolimax reticulatus
1	0	0	1	1	14
2	0	0	0	7	30
3	8	0	0	0	3
4	0	0	0	5	20
5	15	0	0	3	4
6	18	1	0	1	18
7	10	12	0	5	21
8	1	16	0	3	9
9	6	12	0	2	39
TOTALS	58	41	1	27	158

The distribution survey was carried out during the autumn because the known incidence of infection at this period in 25 North Bailey showed no great fluctuations but a steady decline (Fig. 11). The survey could thus be extended over a few weeks in the knowledge that no great changes in incidence were occurring. It was necessary to extend the survey over a period as the number of slugs, especially M. sowerbii, obtained on individual nights was often insignificant.

The results shown in Table 9 and Fig. 14 are based on the total number of slugs collected at each station between September - December 1952.

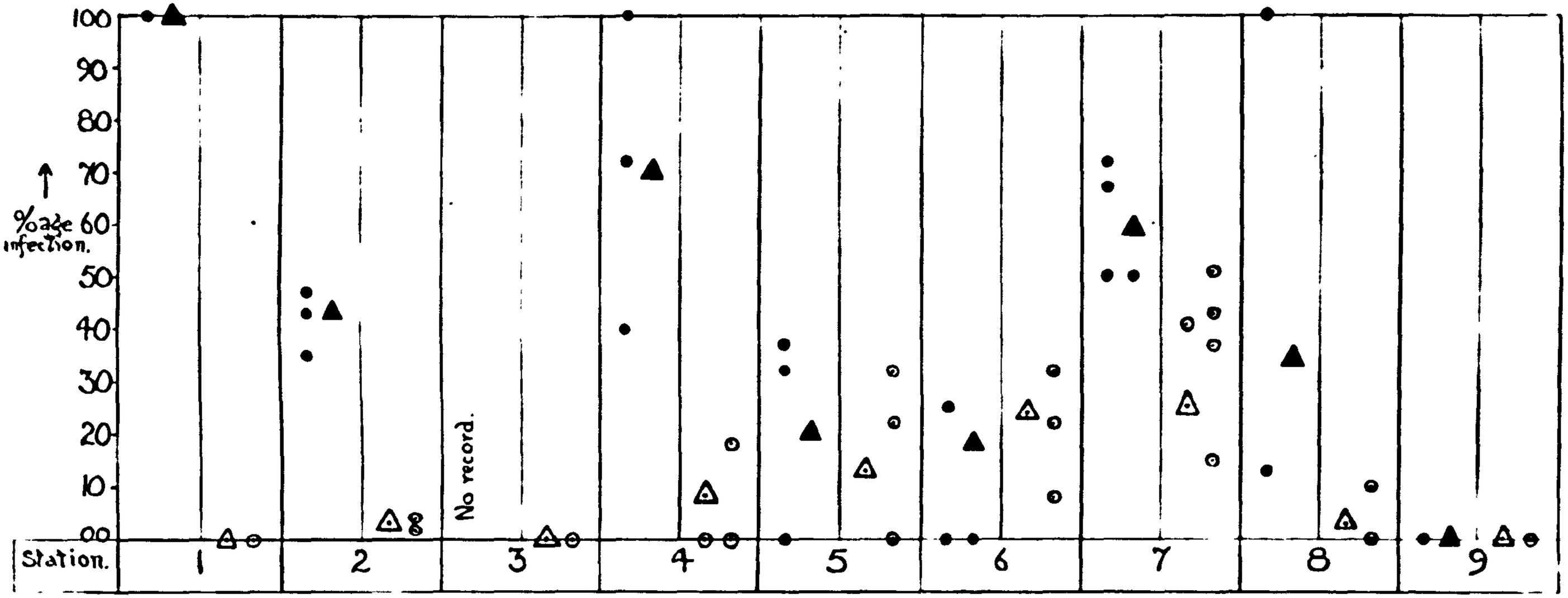
TABLE 9

Incidence of Infection: September - December 1952.

Station.	A. reticulatus		M. sowerbii.	
	Number examined	percentage infection	Number examined	percentage infection
1	14	00	1	100.0[x]
2	60	3.3	14	43.0
3	3	0	0	-
4	67	7.4	20	70.0
5	23	13.0	5	20.0
6	60	23.7	26	18.4
7	82	25.6	48	58.3
8	32	3.1	12	33.3
9	37	0	2	0

[x]The figure of 100% infection for M. sowerbii at station 1 is based on a single slug, and is included only to show that brachylaemids are actually present at this point. None were recorded in the A. reticulatus population

No M. sowerbii was found at station 3.



▲ Milax sowerbii: infection over entire period.

△ Agriolimax reticulatus: infection over entire period.

• Milax sowerbii: individual estimations during period.

○ Agriolimax reticulatus: individual estimations during period.

Fig. 14

Incidence of Infection in A.reticulatus and M.sowerbii

September - December 1952

Fig. 14 shows not only the aggregate estimations of incidence given in Table 9, but also a number of individual estimations; the reason for the rather wide scatter of some of these points is undoubtedly the smallness of many of the individual collections. The mean figures give a truer reflection of the prevailing conditions, and it is to these figures that future reference is made.

The incidence of infection was not constant, but decreased as one proceeded away, in either direction, from 25 North Bailey. Only at station 9 was no infection found. The presence of walls completely traversing the area had no apparent effect on the distribution of the parasite; the latter was certainly not isolated between any such boundaries. The finding of brachylaemids at station 1 indicated that human habitations and their associated small mammals (rats, mice) are not necessarily involved in the life-history.

Table 10 gives data from all known brachylaemid-infested localities; positive cases are denoted by an "x".

Table 10 shows that M. sowerbii was parasitized more often than A. reticulatus, and that infected individuals could live alongside an infection-free A. reticulatus population. Further, in all instances where both species were attacked, except at station 6, the incidence of infection was higher in the M. sowerbii population than in the A. reticulatus.

TABLE 10

Incidence of Infection in M. sowerbii and A. reticulatus.

Location.	Infection recorded in		Inf. M.s.> Inf. A.r.	Inf. A.r.> Inf. M.s.
	M. sowerbii.	A. reticulatus.		
Station 1	x			
Station 2	x	x	x	
Station 4	x	x	x	
Station 5	x	x	x	
Station 6	x	x		x
Station 7	x	x	x	
Station 8	x	x	x	
Bede College	x			
Hallgarth St.	x			
Shincliffe	x	x	x	

From these observations, and evidence concerning the differential effect of the parasites on the two host species (section VI(e)), it is concluded that M. sowerbii is a natural intermediate host of this brachylaemid species, and that tha penetration of the latter into A. reticulatus is a secondary and unnatural phenomenon.

M.sowerbii of less than 150 - 200 mg. were not parasitized; in this respect they resembled the unnatural host species.

V. THE INCIDENCE OF INFECTION DURING 1953.

(a). Introduction.

Although this investigation dates only from September, 1951, the parasite has been known to infest slugs in Durham since 1949. Following the discovery of a trematode sporocyst at 25 North Bailey in October 1949 (section III (f)), a search was made of other slug species of the area, and the first records of brachylaemid metacercariae were established in November 1949 (Cragg and Vincent, unpublished).

During the autumn of 1949, the following localities were searched by the above authors for metacercarially infected slugs: 25 North Bailey, Durham; Bede College; Durham; Science Laboratories, Durham; Old Durham Gardens, Durham; Golf Course Gardens, Durham; Neville's Cross College, Durham; Shincliffe, Co. Durham; Darlington, Co. Durham; and the Moorhouse Nature Reserve, Westmorland (A. ater only). Parasites were found only in M. sowerbii and A. reticulatus from the first two localities.

During the period January 1950 to September 1951, 232 specimens of A. reticulatus from 25 North Bailey were dissected, 38% being parasitized, and 43 M. sowerbii were examined, 56% being parasitized. The records of Cragg and Vincent show the incidence of infection among

A. reticulatus in January 1950 to have been 64%. The value fell rapidly until May (4%), rose sharply during June, and attained a peak (65%) in August. The incidence fell steadily throughout the autumn and winter except for a temporary rise during November, the 1951 spring rise occurring in May.

The records further show that the spring and summer infections were of caudal- and feeding-stage larvae, and the autumn and winter cases of corrugated- and smooth stages. The secondary infection of November 1950 was of caudal stage metacercariae.

Figures of the incidence of infection among M. sowerbii in the area are few, but indicate that this species carried a higher and more constant infection than A. reticulatus; most estimations were 50 - 70%.

These data quoted above show a marked similarity to the findings described in sections III (b), III (c), and IV (b) for the 1951 - 53 period. It is evident that the phenomena described on pp.51-59;73 regarding seasonal incidence, fluctuations, seasonal composition of infections, and the differential susceptibility of the two host species, have all been operative since at least 1949. By 1953 the infection at 25 North Bailey was well established and of at least four years' standing.

(b). The incidence of infection: January - June 1953.

Widespread changes occurred in the parasite-density during spring, 1953. The secondary infection of November 1952 decreased in incidence as expected, but no significant new primary-infection was recorded. By July 1953 the slugs at 25 North Bailey appeared to be free from brachylaemid infection.

The incidences of infection for both host species for the period January - June 1953 are shown in Tables 11 and 12. In these and future tables the 1952 autumnal infection is referred to as the "1952 secondary infection" and the 1953 spring infection as the "1953 primary infection."

TABLE 11

M. sowerbii: Incidence of Infection: Jan - June 1953.

Month.	Number examined.	Percentage infection 1952 secondary.	1953 primary.
Jan.	9	22	0
Feb.	10	0	0
Mar.	11	27	0
Apr.	33	9	0
May.	15	13	0
June.	20	0	0

TABLE 12.

A. reticulatus: Incidence of Infection: Jan - June 1953.

Month.	Number examined.	Percentage infection. 1952 secondary.	1953 primary.
Jan. 28.	32	37	0
Feb. 20.	26	35	0
Feb. 24.	18	28	0
Mar. 2.	12	25	0
Mar. 11.	8	25	12.5
Mar. 12.	16	37	0
Mar. 15.	15	20	13.0
Apr. 1.	30	33	0
Apr. 12.	20	10	0
Apr. 17.	33	33	0
Apr. 21.	19	16	0
May. 7.	6	0	0
May. 19.	38	13	0
June. 4.	30	7	0
June 15.	25	0	0

Table 11 shows that no primary infection was recorded during the period in M. sowerbii.

Table 12 shows that during March there was an indication that a new primary infection was commencing among the A. reticulatus population. This failed to

materialize, however, and no further cases of infection were found during the period. Thus with the extinction of the 1952 secondary infection during May - June, metacercariae appeared to be absent from the area.

In May, when it became obvious that the incidence of infection was not rising as expected, a mass survey of the area shown in Fig. 13 was carried out. The survey was similar to that described on p.69 , the same stations being searched by the same collectors for 45 minutes between 21.00 hrs. and 23.00 hrs. G.M.T. The weather was dry, with no wind and a ground temperature of 16°C.

Table 13 gives a statement of all slugs collected. Thus of 428 slugs collected, A. reticulatus comprised some 63% of the whole, and M. sowerbii about 6%.

The survey was carried out to determine whether the point of greatest incidence of infection had moved away from station 7 (25 North Bailey) to some other region of the area, due possibly to a migration of the vertebrate host. Except for 5 specimens which died and decomposed before examination was possible, all specimens of the two host species were dissected. A number of individuals of the other collected species were also examined, and were found to be free from infection. Further, 50 specimens of A. ater were examined for sporocysts and cercariae, none being found.

The results of the examinations of M. sowerbii and

TABLE 13

Spring Slug Population: 6 May, 1953.

Station.	Number present in 45-minute collection.						
	Arion ater	Arion hortensis	Arion circumscriptus	Limax maximus	Milax gracilis?	Milax sowerbii	Agriolimax reticulatus
1	2	0	0	0	0	0	3
2	12	17	0	8	1	6	74
3	26	0	0	2	0	2	7
4	4	2	0	0	0	0	44
5	16	3	1	0	0	7	19
6	2	6	0	0	0	0	8
7	0	0	0	0	0	4	6
8	12	10	0	3	0	7	68
9	3	2	0	0	0	1	40
TOTALS	77	40	1	13	1	27	269

A. reticulatus are given in Tables 14 and 15. Table 14 showw the total figures for these species, and Table 15 the incidence of infection at the individual stations.

TABLE 14.

Infection among Host Populations: 6 May, 1953.

Host Species.	Number examined	Number infected. 1952 secondary	1953 primary.
M. sowerbii.	27	3	3
A. reticulatus.	264	19	10

Thus of 291 possible hosts examined, only 35 were carrying metacercarial infection, and of these only 13 were 1953 primary infections. One specimen of A. reticulatus harboured both a 1952 secondary- and a 1953 primary infection.

Table 15 shows that in A. reticulatus primary infections were recorded at only three stations, the maximum incidence being 7% at station 4. The data for M. sowerbii are based on considerably fewer specimens, but still show infection to have been much rarer than expected.

Further collections at later dates showed that apparently no new infection arose at any point, and that the infections recorded in Table 15 did not increase in intensity. In June only isolated cases of infection could be found.

TABLE 15

Distribution of Infection: 6 May, 1953.

Station	A. reticulatus.			M. sowerbii		
	Number examined	percentage infection 1952 secondary	1953 primary	Number examined	percentage infection 1952 secondary	1953 primary
1	3	0	0	0	-	-
2	74	1.0	5.0	6	0	17.0
3	7	0	0	2	0	0
4	40	5.0	7.0	0	-	-
5	19	21.0	0	7	29.0	29.0
6	8	0	0	0	-	-
7	6	0	0	4	0	0
8	67	14.0	4.5	7	14.0	0
9	40	7.0	0	1	0	0

(c) Incidence of Infection: August - December, 1953.

Collections were continued at 25 North Bailey throughout the summer and autumn. The monthly totals and infection-levels for both host-species are given in Table 16. The month of November has been split into two sections because the incidence of infection in M. sowerbii showed a significant change during the month (see section VI (e) p.112).

TABLE 16

Incidence of Infection: August - December, 1953.

Month.	A. reticulatus.		M. sowerbii.	
	number examined	percentage infection.	number examined	percentage infection.
Aug.	12	17	6	17
Sept.	49	12	28	61
Oct.	25	20	5	40
Nov 1 - 15.	6	0	80	40
Nov 16-30.	43	9	53	21
Dec.	45	16	17	29

Table 16 shows that the autumn was characterized by a reappearance of brachylaemid parasites in the natural host, M. sowerbii. The incidence of infection rose to 61% in September and was maintained at a fairly high level until the end of November when a steep

decline occurred due to a lethal effect on the host (see section VI (e) p.111).

There was no comparable resurgence of infection in A. reticulatus, intermittent cases being recorded throughout the year; there appeared to be no significant fluctuations in incidence.

At the end of November, three infections of caudal stage metacercariae were recorded from M. sowerbii. This was the 1953 secondary (autumnal) infection, and was not recorded in A. reticulatus.

Table 11 (p.77) showed that up to June, no primary infection was found in M. sowerbii. The re-infection of this species appeared to be in August (Table 16), but the larvae found then were all corrugated- and smooth stage individuals. The infection between August to December thus appeared to be quite normal, and the origin of these metacercariae remains unsolved.

The infections recorded from the A. reticulatus population between August to December were also of corrugated and smooth stage larvae, but a low incidence of caudal stage infection had been previously recorded in March (Table 12, p.78) in the area.

The problems raised by these abnormal phenomena are referred to in the Discussion.

VI. THE EFFECT OF THE PARASITE ON THE INTERMEDIATE HOST.

Probably because of their unencysted condition, these brachylaemid cercariae exert a profound pathological effect on their hosts; similar effects have not been previously described in this or other species.

(a). Materials and Methods.

Slugs were kept under normal laboratory conditions in large glass aquarium tanks containing about 5 cms.of moist soil. They were fed on lettuce, cabbage, or carrot, and occasionally meat-lights. All food was removed before decomposition set in. Oat seedlings were grown in the cultures to purify the air. Cultures were maintained of both healthy and infected individuals.

For daily observations the animals were transferred to lamp-glasses containing moist soil; the food was changed daily.

Eggs were collected from the cultures as required, and placed on moistened filter-papers in petri-dishes, watch being kept for fungal growths. At room temperature, hatching occurred 3 - 4 weeks after laying. Eggs were also placed in Plaster-of-Paris containers set in bowls containing a shallow layer of water to ensure a constant supply of moisture (Ulmer 1951). Slug eggs failed to hatch under these conditions.

Recently-hatched slugs were maintained in the petri-dishes with the eggs for the first 4-5 days of life, and then transferred to aquarium tanks containing moist sterilized soil. Pure, healthy cultures were thus established.

The renal tissue was fixed in Bouin's Picro-Formol mixture, 70% alcohol, 4% formalin, 8% formalin, and formol alcohol. Best results were with Bouin's Fluid for 24 hours. Stains employed were Mallory's triple Connective Tissue Stain, Heidenhain's Iron Haematoxylin, Delafield's Haematoxylin, and Erlich's Haematoxylin. The most successful method was a modification of Mallory's technique which is recorded in Appendix II.

The Hollande Modification of the Courmont-André Method (Glick 1949, and Appendix II) was used as a test for uric acid in renal tissues. This should have resulted in uric acid appearing black in colour. The actual result was a grayish-black colour which often took some hours to appear and which sometimes later faded and disappeared. The results were obviously unsatisfactory.

The Mirexide Test (Glick 1949) was also used. This also proved unsatisfactory owing to the powerful action of the nitric acid employed.

(b). The Effect on the Kidney.

(i) The Healthy Kidney.

In Agriolimax reticulatus the renal organ is a small, almost spherical body, about 2 mm. in diameter. It is ochreous yellow in colour and lies immediately beneath the mantle cavity, slightly posterior and dorsal to the pericardium and the heart.

In Milax sowerbii it is a correspondingly larger structure (about 4 - 5 mm. in diameter), flattened dorso-ventrally and in the form of a hollow circle. It encircles the heart and pericardium. In both host-species the kidney is enclosed in a thin, sheath-like membrane, and communicates with the pericardium via the renopericardial canal, and with the exterior via the thin-walled ureter.

Histologically, the kidney is of a connective and vascular nature, the internal wall being thrown into a number of folds or lamellae which project into and completely fill the lumen of the organ. The main mass of the kidney is composed of a mass of well-defined epithelial cells which appear to be relatively thick-walled. The main volume of each cell is composed of a vacuole, and the nucleus is not prominent; it may appear as a darkly-staining mass close to the lamella-boundary of the cell, but often there is no evidence of the structure. Many of the cells contain granular

inclusions, some of which are thought to be of uric acid. The whole renal organ is permeated by a plexus of blood vessels.

The structure of the normal, healthy kidney of both host species is shown in Figs. 15 and 18; The dark lines represent the renal lamellae.

(ii) The Parasitized Kidney.

Infection by caudal stage larvae appeared to be of little or no pathological significance. Kidneys supporting up to 100 such parasites were observed to exhibit a normal histological appearance. It is possible that the physiology of the organ was disturbed but that the histological changes were not evident until later.

The onset of feeding-stage metacercariae, however, produced large-scale anatomical changes as well. The parasites lay freely among the lamellae or became attached to the latter (Fig. 8). A general breakdown of the host's renal tissue then ensued, the results of which are shown in Figs. 16, 17, 19, and 20.

Fig. 19 shows that the general necrosis originated in that area of the kidney immediately containing the parasites; it later spread throughout the whole organ, even when the larvae inhabited only a part of it, until the appearance was that shown in Figs. 16 and 17.

The lamellae disintegrated and disappeared, and the cells became isolated from each other and heavily laden with concretions of varying appearances. At a later stage the cell-walls broke down, and the organ became a mass of necrotic cell-dêbris, isolated remnants of lamellae, and uric acid and other granular materials. Fig. 20 illustrates a further stage, in which the major part of the disorganized mass had disappeared, and left only the outer connective tissue shell of the kidney surrounding the parasites, and scattered patches of detritus.

The time taken to reach this condition varied in different individuals; when the kidney was disorganized to the extent shown in Figs. 16, 17, and 20, the parasites were usually in the later phases of the corrugated stage, although some were still feeding.

The presence of various materials within the gut of brachylaemid metacercariae has been noted by several workers. Leidy (1850) referred to "...organic cells with granular contents." Hoffmann (1899) mentioned "Nierenepithelien und Harmoconcrementum" filling the crura of metacercariae from the kidney. Krull (1935) in Panopistus pricei found "...kidney-tissue"in the gut of young larvae confined to the kidney but noted that the caecae of those individuals in the pericardium were clear. Balozet (1937) stated that "... granulations alimentaires" occurred in the guts of

metacercariae of B. suis, while Dollfus (1938) noted that young larvae from Helicella obvia contained renal tissues within the gut, but that in older stages the gut became empty. Ulmer (1951) recorded the presence of "...typical kidney concretions" in the gut of an unidentified metacercaria from Angiuspira alternata.

In the present brachylaemid species, the dense mass which filled the gut of the feeding-stage larvae consisted of necrotic kidney tissue and its inclusions (Fig. 21). It appeared then, that following an apparently benign caudal stage, the feeding stage larvae caused a breakdown of host tissues and ingested the resulting dêbris. At that stage the kidney assumed a necrotic appearance even on the external surface. The corrugated- and smooth-stage larvae lay among the detritus which often contained a number of clear spaces (Fig. 20) and presumably did no further harm to the host.

These histological changes occurred equally in both host-species; the physiological changes appeared to differ, however (section VI (e) p102-113).

PLATE 2.

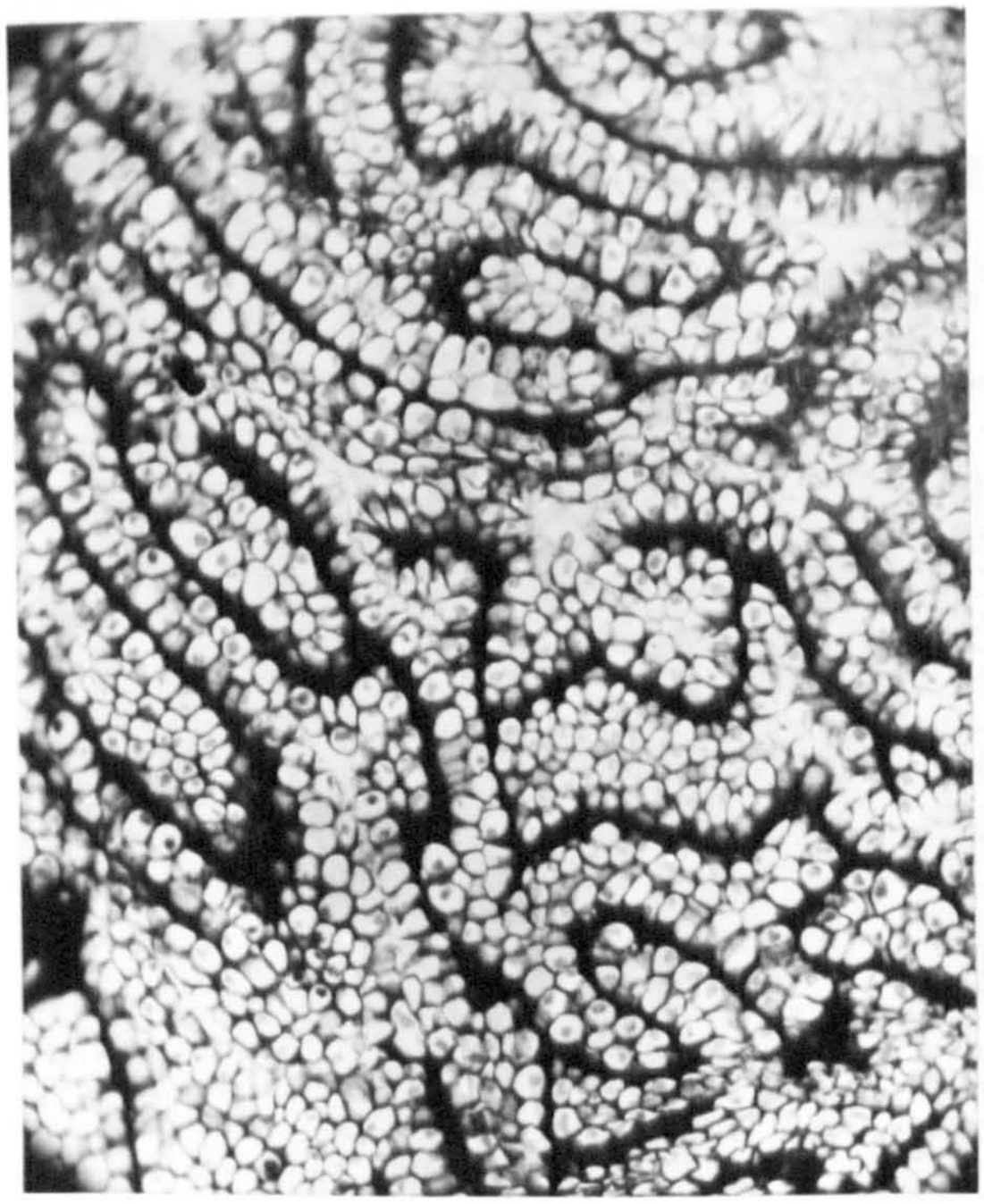


Fig.15.
M.sowerbii:Healthy Renal Tissue. x100.

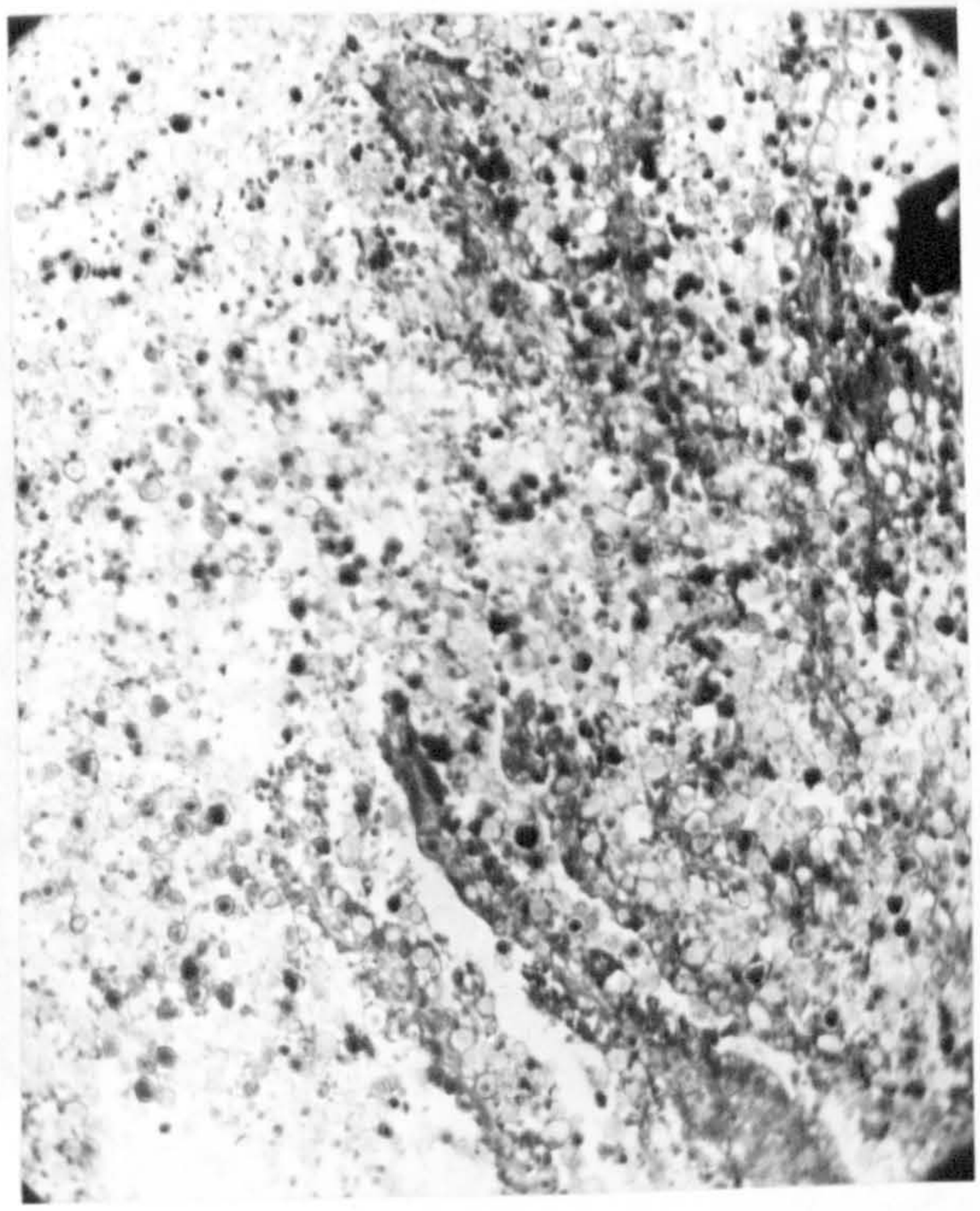


Fig. 16.
M.sowerbii:Infected Renal Tissue. x100.

Fig. 17.
M.sowerbii:Renal Tissue with Parasite 'in situ'. x100.

PLATE 3.

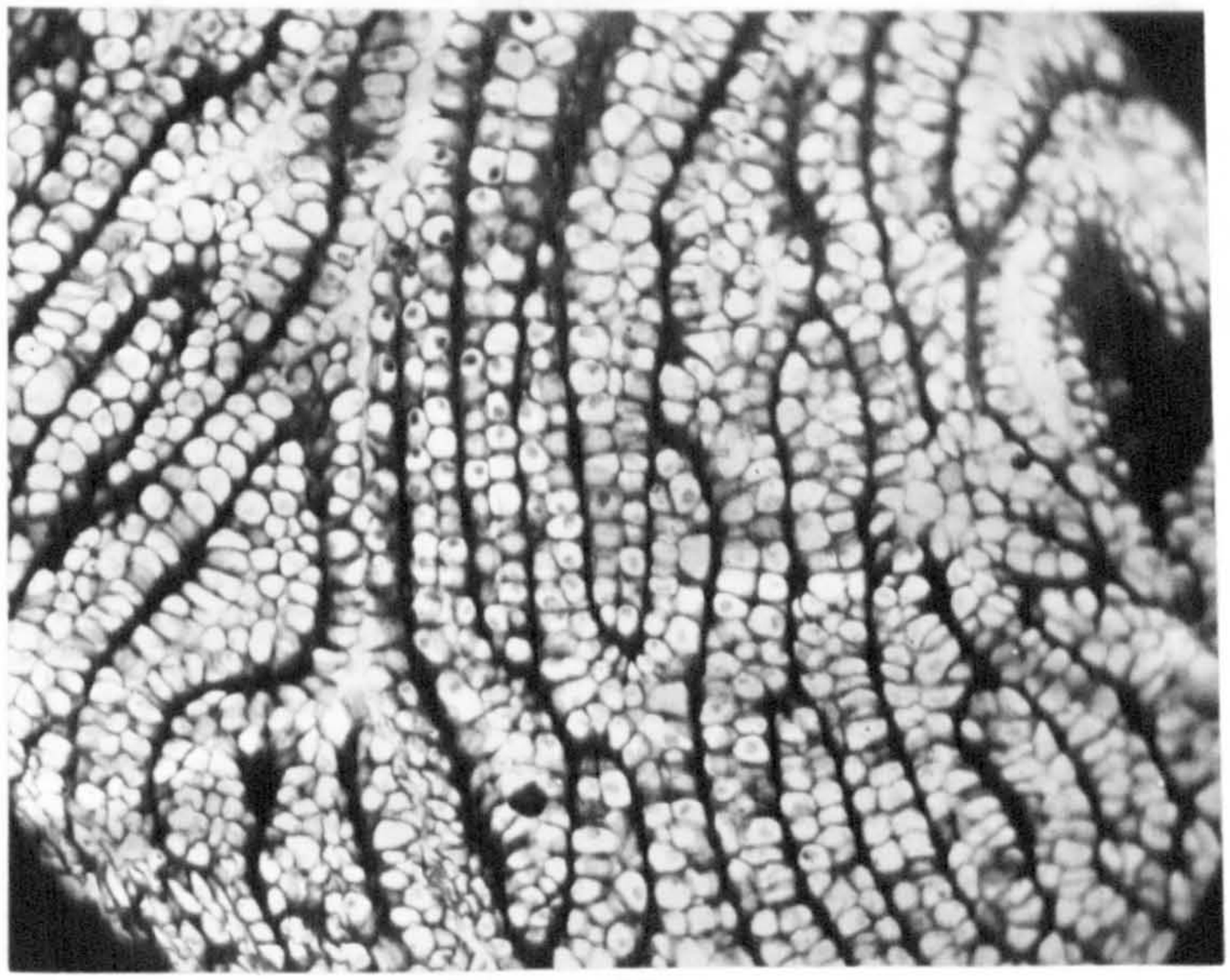


Fig. 18.

A.reticulatus: Healthy Renal Tissue. x100.

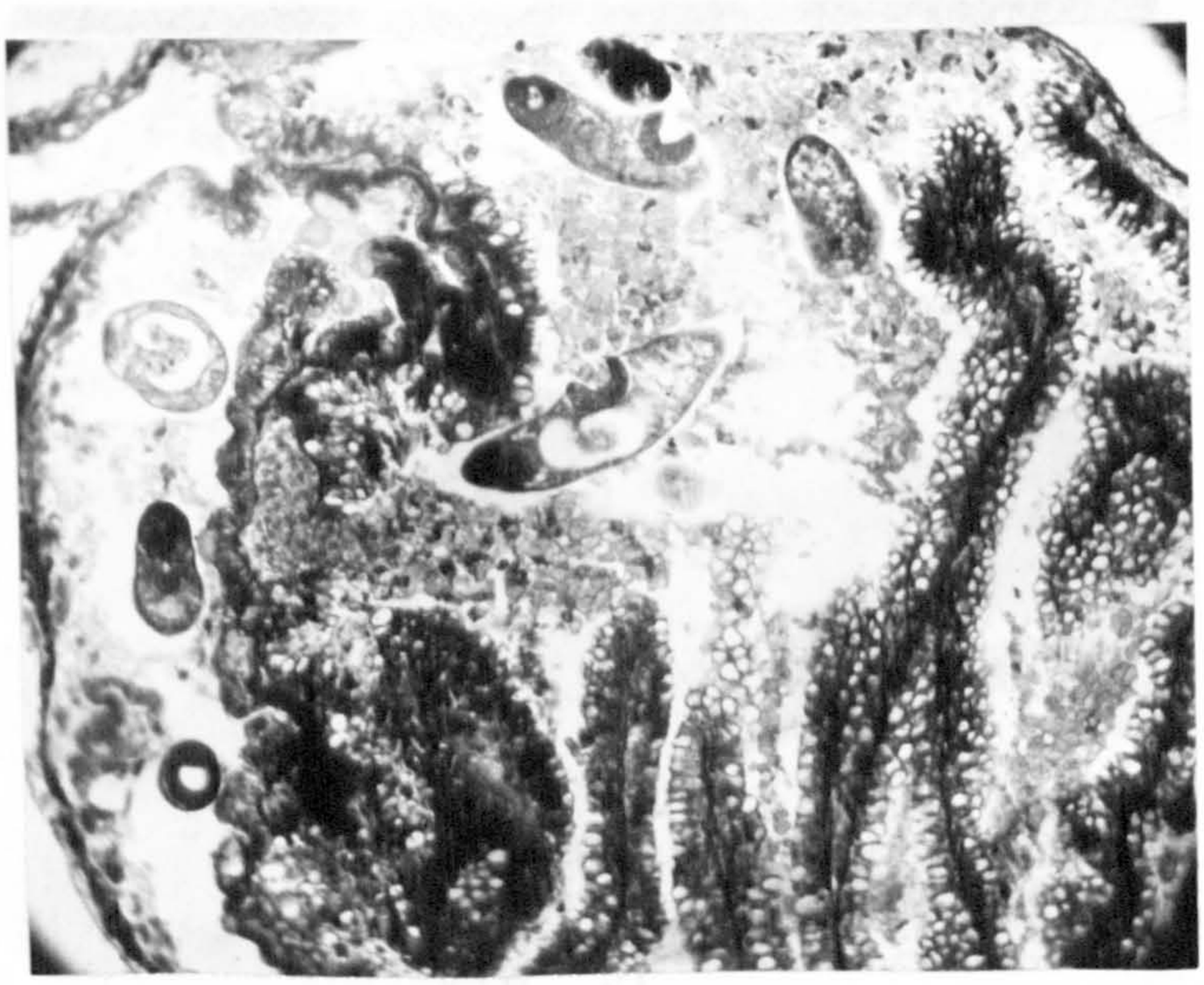


Fig. 19.

A.reticulatus: Infected Renal Tissue with Parasites 'in situ'. x50.

PLATE 4.

Fig. 20.

A.reticulatus: Renal Tissue in final stages of necrosis. x100.

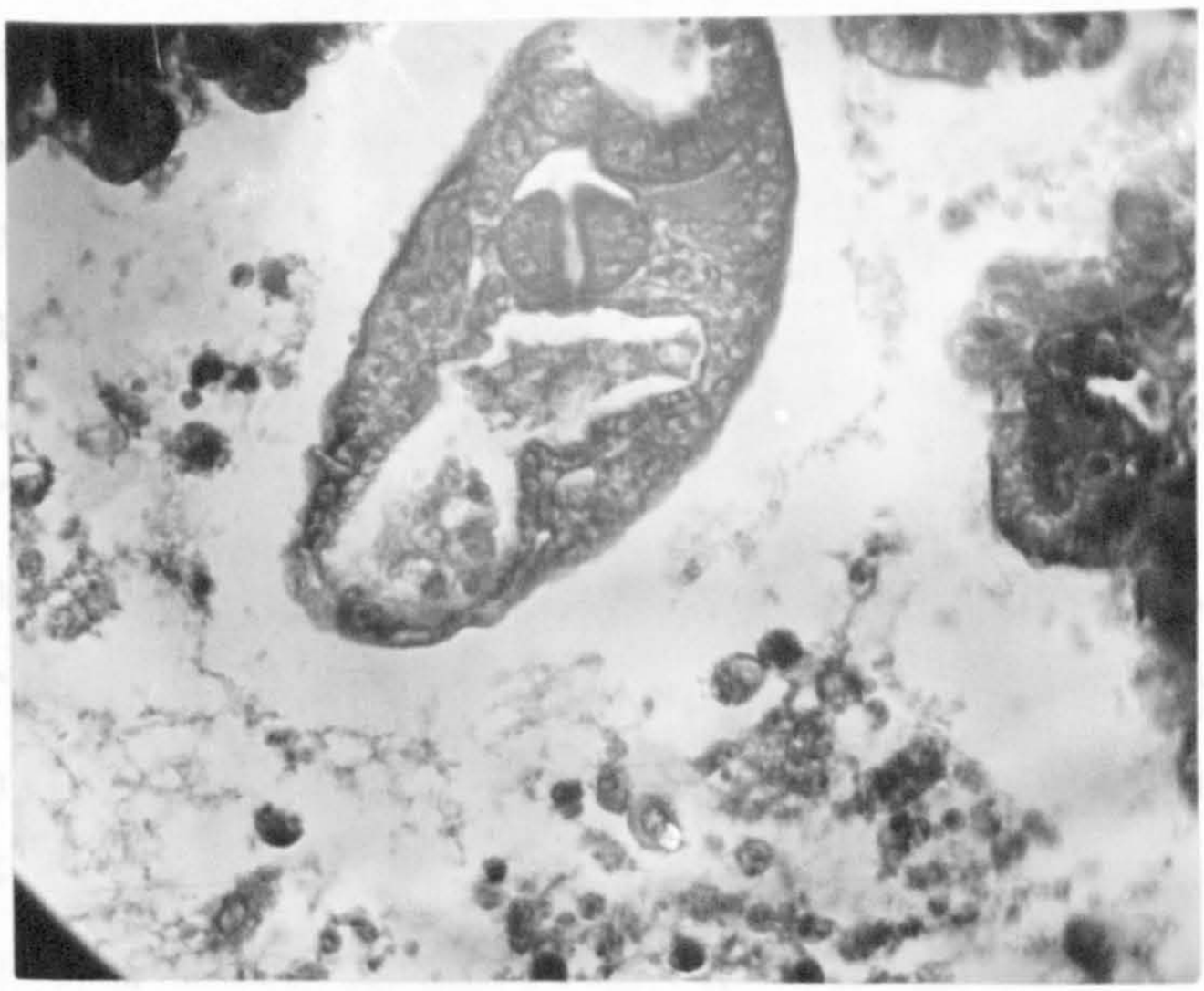


Fig. 21.

A.reticulatus: Metacercaria with food in gut. x400.

(c) The Effect on the Ingestion of Food.

A. reticulatus from 25 North Bailey were maintained in lamp-glasses as described (p.85). The initial number of animals in each culture was usually 10, and any dying during the observations were dissected to determine whether parasitized. At the end of the observations all survivors were killed and also dissected, thus enabling the percentage infection of each culture to be determined. A weighed amount (3 - 4 grams) of dry lettuce leaf was provided daily in each culture, removed after 24 hours, reweighed, and the number of animals in the culture noted. Thus, knowing the total amount of food consumed by all slugs during the previous 24 hours, the average consumed by each individual during the same period was determined.

The results are given in Tables 17 and 18. The figures show the average amount (gms.) of food ingested per slug per 24 hours.

The individuals of each culture were all of a similar weight to avoid great variations in the feeding capacity of individual animals in a culture.

A "control culture" was maintained without animals, but which received food daily. Changes in weight due to causes other than ingestion (eg. evaporation of constituent water) were thus determined. In no case did the control food gain weight; the greatest loss

was 2.4% of the initial weight in 24 hours, the smallest loss was 0.9% and the average loss 1.5%. These figures are not included in Table 18 as they do not materially affect the result; they are included in the smaller Table 17 merely as an example.

Tables 17 and 18 show that food was consumed at a higher rate in those cultures with a low incidence of infection than in otherwise comparable cultures with a higher incidence. Table 18 shows that only in observation 15 did the slugs of cultures 4 or 5 (90% infection) consume as much as the similar-sized animals of culture 3 (20% infection).

TABLE 17

Average Daily Consumption of Food (gms/24 hrs): A. reticulatus.-(1).

Observation.	3.0-4.0 gms. in weight.		%age change in weight of Control food.
	Culture 1. 70% infection.	Culture 2. 22% infect.	
1	0.139	0.228	-0.92
2	0.050	0.187	-2.40
3	0.064	0.156	-1.60
4	0.067	0.148	-1.10
5	0.080	0.145	-0.92
6	0.085	0.176	-0.99

TABLE 18.

Average daily Consumption of Food (gms/24 hrs):
A. reticulatus - (2).

Obs.	2.0 - 4.0 gms. in weight.			4 - 6 gms wt.
	Culture 3 20% Inf.	Culture 4 90% Inf.	Culture 5 90% Inf.	Culture 6 100% Inf.
1.	0.275	0.256	0.144	0.193
2.	-	0.186	0.130	0.198
3.	0.200	0.130	0.084	0.243
4.	0.161	0.156	0.106	0.144
5.	0.192	0.074	0.132	0.114
6.	0.279	0.072	0.077	0.156
7.	0.199	0.078	0.083	0.094
8.	0.209	0.090	0.032	0.127
9.	0.165	0.094	0.064	0.068
10.	0.190	0.042	0.025	0.025
11.	0.170	0.027	0.120	0.100
12.	0.195	0.030	0.083	0.046
13.	0.170	0.020	0.073	0.032
14.	0.142	0.025	0.083	0.042
15.	0.064	-	0.110	0.027

Consumption in Culture 3 was often seven times that of Cultures 4 and 5. Further, only once did the slugs of 4.0 - 6.0 gms weight in Culture 6 (100% infection) consume more than the smaller animals of Culture 3 (20% infection).

The inference is that the parasitization lowers the rate of ingestion of food of the host. The data, however, are based on mass-culture conditions only. Observations on single hosts were started, the hosts also being weighed daily to determine the effect on growth. Difficulties arose concerning the weight records of the slugs, and the work was left until that described in section VII was done. Unfortunately the great reduction in the parasite density among A. reticulatus in 1953 (section V) prevented a resumption of the feeding experiments.

(d) The Effect on Fecundity.

(1) Effect on Egg Production.

The investigation into this effect was again carried out on a number of hosts under mass-culture conditions. The reduction in the parasite-density among A. reticulatus in 1953 (section V) prevented observations being made on single individuals.

The results obtained indicated that egg-production in A.reticulatus was reduced by brachylaemid infestation.

A number of cultures were maintained in lamp-glasses as described. Each contained approximately 10 animals, with a different proportion of healthy individuals, as determined later by dissection.

Eggs were removed from the cultures daily, and the number of living slugs noted. In order to collect all the eggs laid, the soil of each culture was examined weekly in an open tray. Observations were continued for 25 days.

The results are given in Table 19 and Fig. 22, and are expressed in the following way. By keeping a daily record of slugs dying in culture, at the close of the experimental period it was known for how long each slug in each culture had lived; thus the average length of life of the slugs in each culture was calculated. The total number of eggs laid in each culture was known from observation.

Knowing, for each culture, the initial number of slugs, the average length of life of these slugs, and the total number of eggs laid, it was possible to calculate the average number of eggs laid per day per slug.

TABLE 19

Egg - Production of A. reticulatus.

Culture.	Number of Slugs.	Mean Length of life (days)	Total No. of eggs laid	%age Healthy	Mean No. of Eggs/ Day/Slug.
1	11	12	20	0	0.150
2	10	12	50	10	0.420
3	10	13	91	10	0.700
4	10	13	110	40	0.850
5	13	18	150	62	0.640
6	10	23	356	80	1.550
7	9	18	390	78	2.390
8	10	14	230	0	1.640

Fig. 22 shows that in cultures 1 - 7 there was a rise in the average number of eggs/day/slug corresponding to the rise in the percentage of healthy individuals in the cultures. Culture 8, however, is at variance with this general trend, having a mean egg-producing capacity approximately ten times that of culture 1, although neither culture contained any healthy individuals.

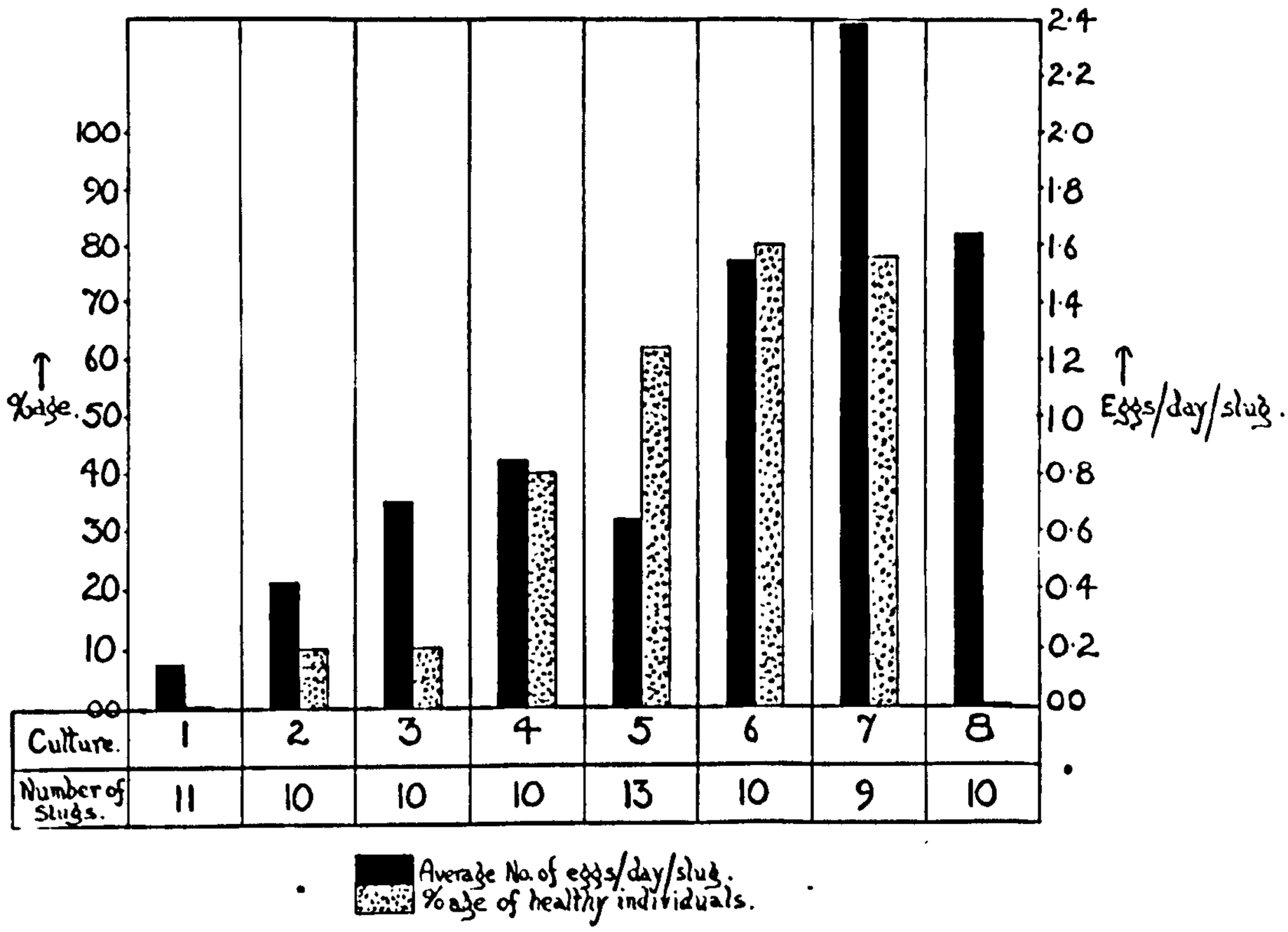


Fig. 22.

A.reticulatus: Egg production and Incidence of Infection.

(ii) Effect on Egg viability.

Attempts to determine the viability of eggs laid by both healthy and infected A. reticulatus were hindered by the persistent growth of fungae on the eggs before hatching. Because of the unreliability of normal methods (p.85), eggs were placed on filter-paper moistened with tap-water, boiled tap-water, or distilled water, and also on a soil surface kept moist and drained. None was really satisfactory.

The best method was by washing the eggs in distilled water and placing them in a closed petri-dish on filter-paper slightly moistened with tap-water and from which all excess liquid had been pressed out with a cloth. One or two drops of water every 2 days kept the eggs moist.

TABLE 20

Egg - Viability in A. reticulatus

Culture.	Percentage Infection.	Number of eggs observed.	Percentage hatched after 4 weeks.
1	22	290	14.0
2	40	170	35.0
3	60	100	15.0
4	100	105	41.0

Observance of these conditions, however, failed to prevent the loss of many eggs; previous (unpublished) work has also indicated the uncertainty of hatching slug eggs in a quantitative manner in the laboratory. A specimen of the results obtained is shown in Table 20.

Table 20 shows that the incidence of hatching was often low and irregular, and apparently unrelated to the incidence of infection among the parents.

(e) The Lethal Effect.

The effect of the parasitism on the longevity of the hosts was investigated in M. sowerbii and A reticulatus from 25 North Bailey, Durham.

Slugs were collected during the spring and summer of 1952 and maintained in cultures as described. The animals in each culture were all of similar size, and in the lamp-glasses were 10 in number. Observations on larger numbers in aquarium tanks were discontinued owing to the periodic disappearance of individuals; such individuals were omitted from all calculations. The cultures were maintained at 15° - 21°C, and inspected daily. Food was changed daily and dead individuals removed and examined for parasites. The cultures were maintained for up to 3 months, and finally all

survivors killed and the incidence of parasitism determined by dissection.

The results for A. reticulatus are given in Figs. 23, 24, and 25; those for M. sowerbii in Figs. 26 and 27. All figures are based on the 25-day period after the cultures were established; this allowed comparisons between cultures to be made, and reduced (but not eliminated) the chances of including deaths due to causes other than parasitism (eg. senescence).

(i). Effect on A. reticulatus.

Cultures 11, 12, and 13 (Fig. 23) were obtained from areas with very small or no incidence of parasitism, and thus acted as controls against the other cultures which were all from 25 North Bailey.

Fig. 23 shows the correlation between the incidences of infection and mortality in A. reticulatus. The percentage mortality and percentage infection were closely allied; cultures 12 and 13, which were free of parasites, showed only one death out of 20 slugs, while cultures 1 - 6 were all infected at an incidence of over 80%, and the lowest mortality recorded was 77% in culture 6. Culture 11 (20% infection) had a mortality of 10%. With ten slugs per culture, the minimum variation possible is 10%, and the figures shown in

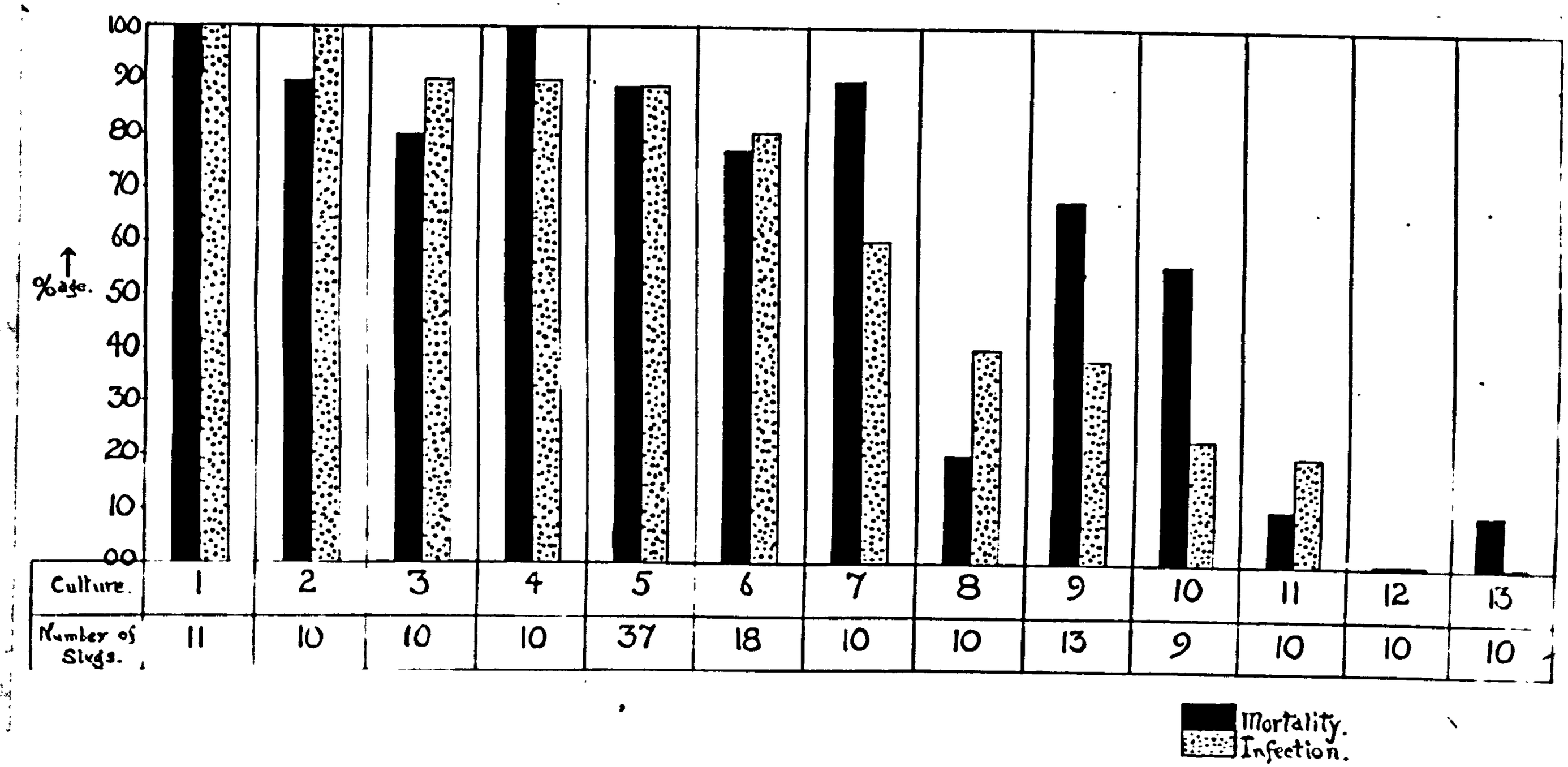


Fig. 23

A.reticulatus: Incidence of Mortality and Infection

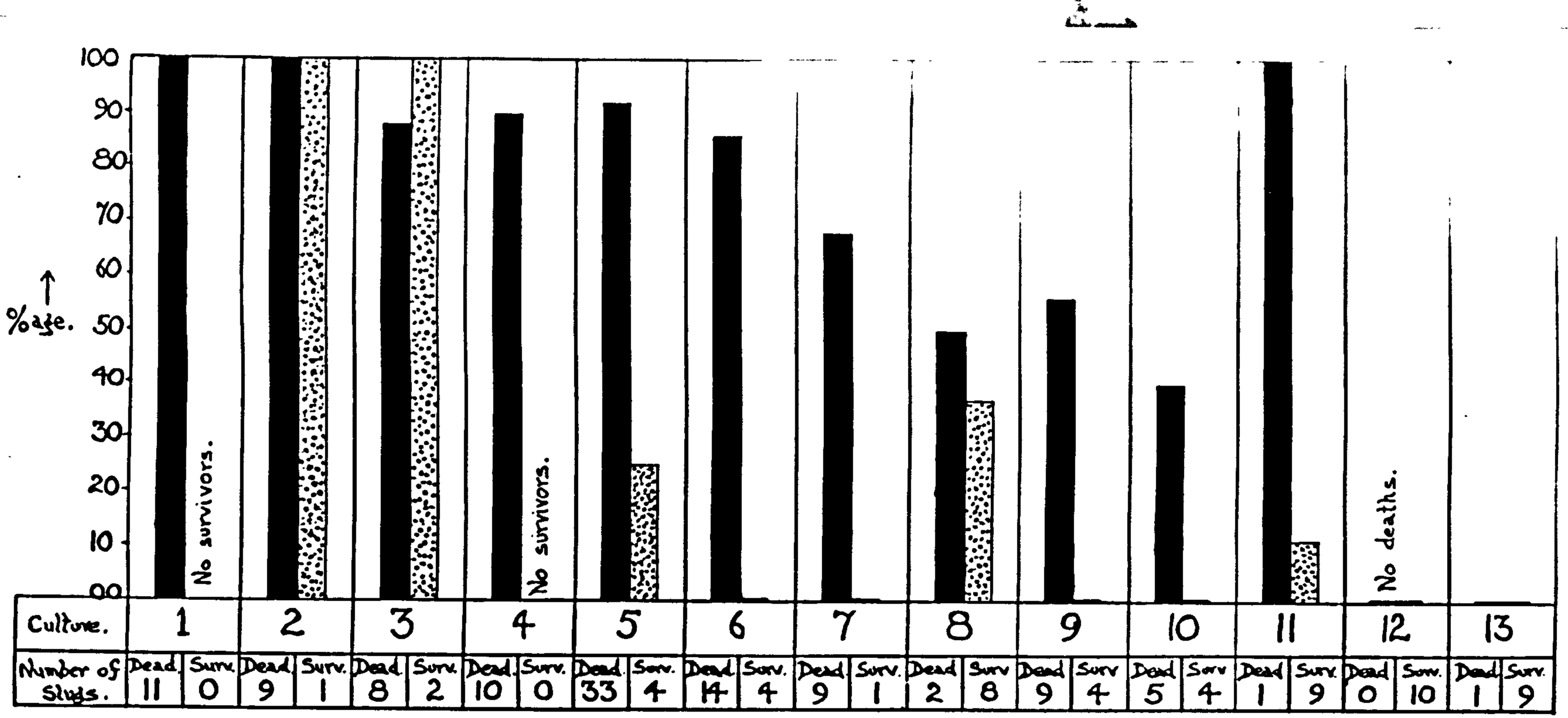


Fig. 24

A.reticulatus: Incidence of Infection among a) Deaths, and b) Survivors.

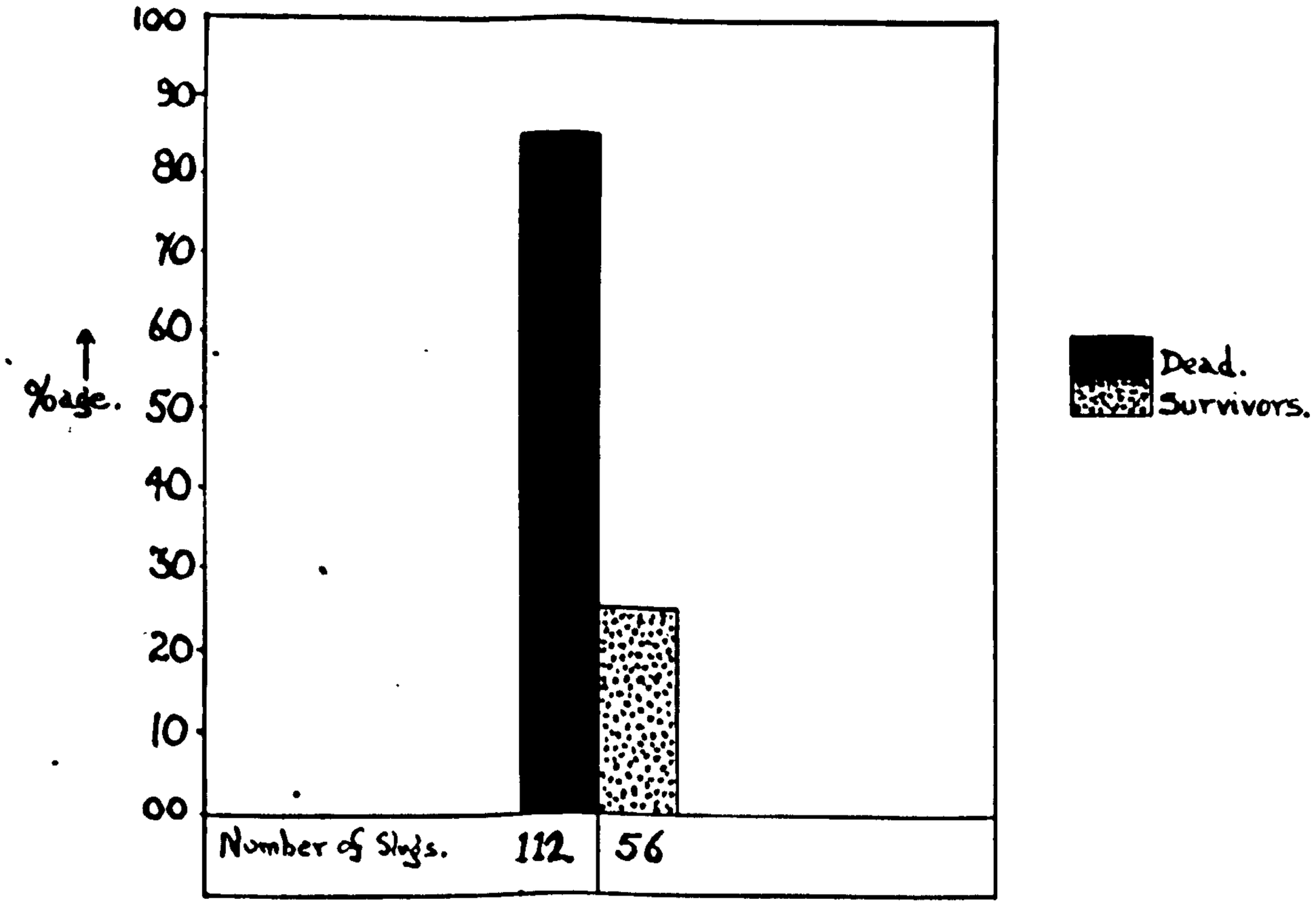


Fig. 25.

A.reticulatus: Incidence of Infection among a)total deaths, and b)total survivors.

Fig. 23 thus show a remarkable correlation.

Fig. 24 confirms the connection between infection and mortality. In each culture, the percentage infections among all animals dying during the 25 days, and all animals surviving the same period, were calculated separately. It is seen that in cultures 1 - 6, and 11, over 80% of the individuals which died were harbouring an infection, the minimum incidence in all cultures being 40% (Cult. 10). In all cultures which contained survivors except 2 and 3, the majority of the survivors were quite healthy. In six cultures there was no infection among the survivors, and the maximum incidence recorded in others was only 37% (culture 8).

Cultures 2 and 3 (Fig. 24) appear to be in contrast to the others; this however is true only for the particular period covered by the histograms. After some further time, the three survivors of these two cultures died. Thus at a later date cultures 2 and 3 would have shown "no survivors" and not 100% infection among survivors as in the present figures. This would comply with the general scheme.

Because of the small number of survivors in some individual cultures, the values expressed in Fig. 24 have been totalled to give a statistically more accurate statement. Fig 25 shows the incidence of infection among all slugs dying in all cultures for the period, and among all slugs surviving in all cultures. Of the total which died (112), 85% were

infected, and of the total which survived (56), 25% were infected, and some of these died at later dates.

It is concluded that the parasitization of A. reticulatus leads to a mortality of the hosts comparable with the incidence of infection in the population.

(ii) Effect on M. sowerbii.

Cultures of M. sowerbii, under similar conditions to those of A. reticulatus, gave results in contrast to the latter.

Fig. 26 shows that although cultures 1 - 4 supported an incidence of infection between 50 - 75%, the incidence of mortality was in no way comparable, the maximum recorded being 20% in culture 3. The perfectly healthy culture 5 had a death-rate comparable with that of the heavily infected cultures.

Cultures of M. sowerbii were maintained for some months, the number of survivors being noted at intervals. The survival data are given in Fig. 27. The number of animals remained fairly constant during the summer and early autumn, despite the high levels of infection present. Cultures 1 and 4 were terminated while the numbers were still constant. Culture 2 (73% infection) remained constant until October, when

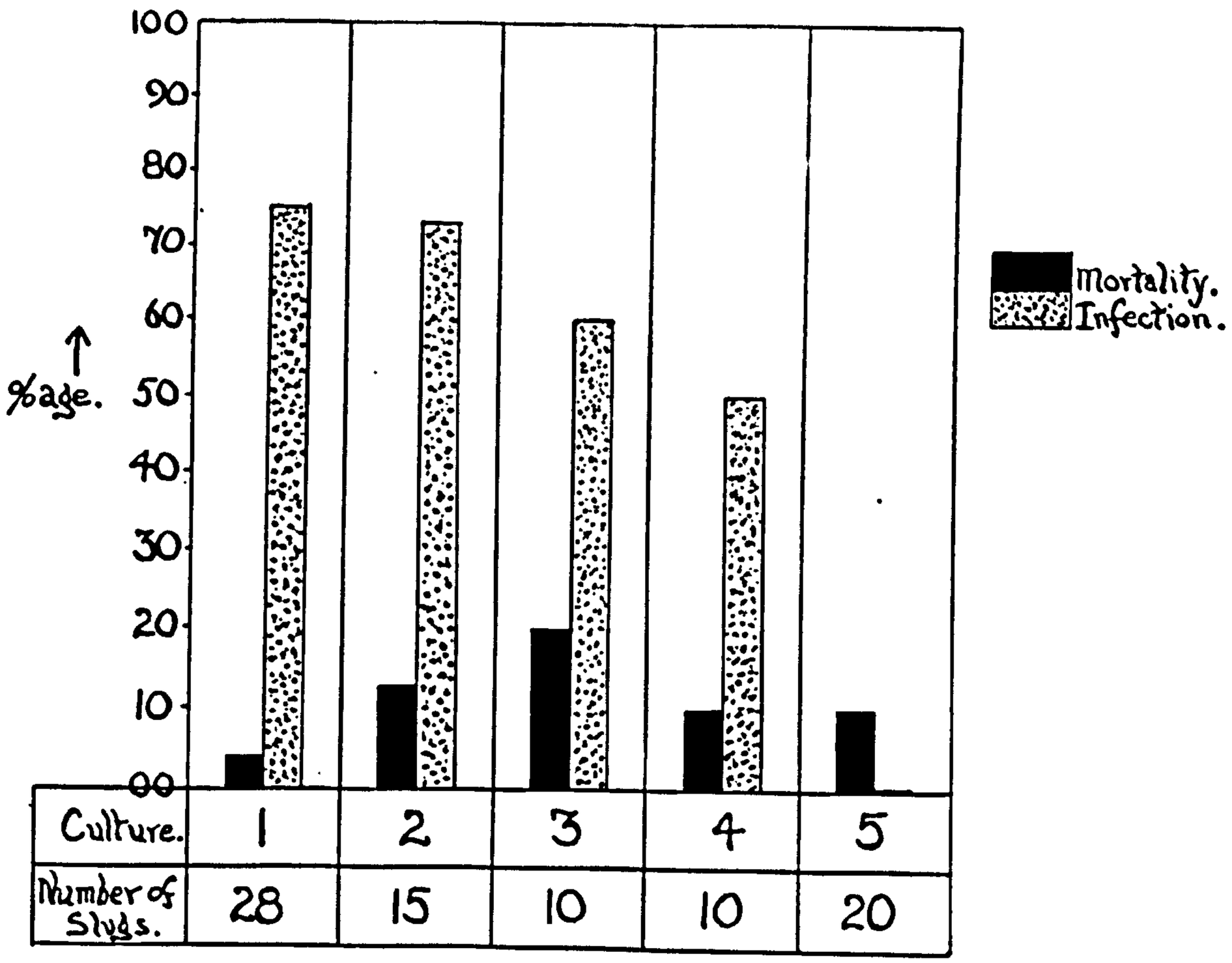


Fig. 26.

M.sowerbii: Incidence of Infection and Mortality.

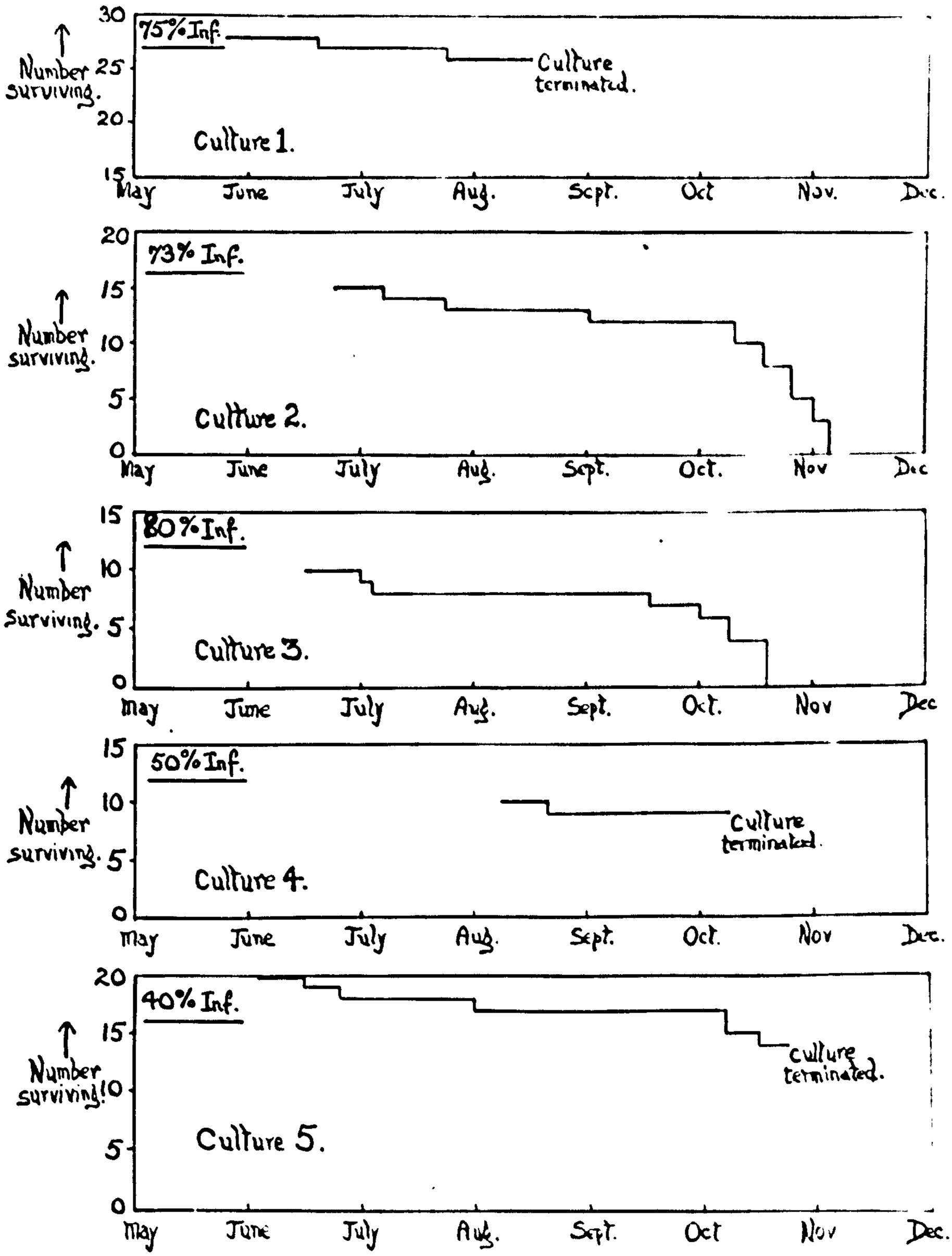


Fig. 27.

M.sowerbii: Longevity of Infected Cultures.

when a steep decline led to its extinction during November; culture 3 showed a similar dying out between September and October. Culture 5 (40% infection) was also showing an increased mortality at the time of its termination in late October. Cultures of healthy M. sowerbii from areas other than 25 North Bailey have been kept successfully throughout a number of winters without any evidence of a high autumnal mortality, such as is indicated in Fig. 27.

It is concluded then that M. sowerbii has greater powers of resistance to the parasites than has A. reticulatus, and can survive throughout the summer with a renal organ broken down to the extent shown in Fig. 16 (p.91). The lethal effect on this host is delayed for some months.

(iii) The Natural Intermediate Host.

The differential effect of the parasites on the two host-species supports the conclusion that M. sowerbii is the natural intermediate host of this brachylaemid (p.74). The winter mortality of infected M. sowerbii in cultures has also been observed in the field. Table 5 (p.53) shows a decrease in the incidence of infection during the winters of 1951/52 and 1952/53.

These figures however are based on small samples, and more reliable evidence is found in Table 16 (p.83); this shows a steep drop in infection-level during the second half of November 1953, and is based on sufficient specimens to be regarded as significant field evidence in support of the culture observations. This autumnal decrease in infection-level in M. sowerbii is due to the death of parasitized individuals and is comparable with the similar decrease recorded in A. reticulatus during mid-summer (p.51).

It is well known that a parasite does not intentionally cause fatal injury to its natural host and thus terminate its own life as well. In the present case it appears that the metacercariae cause fatal injury to any hosts in which they are present, but that only in an unnatural host does death occur before the parasites reach maturity. In the natural host, death is delayed until the larvae have reached the infective stage and vertebrates have been exposed to infection to some time. Further, by November - December many vertebrates are hibernating or inactive.

Host-deaths during the winter are then immaterial to the parasites.

(f) Other Effects.

No further effects have been observed.

The colour of the hosts remains normal during infection except for the immediate pre-death period, and the activity as measured by the actograph described in Appendix I is no different to that of healthy slugs except in the 48 hours preceding death, when the host often lies moribund.

The metacercariae lie only in the renal tissues and slugs dying as a result of infection exhibit a normal histological appearance in all other organs.

The weight and growth rate may be affected by the reduced intake of food during infection, but it was not possible to study this (p.97).

The water-relations of the hosts may also show changes as a result of the parasitism, but a clearer understanding of the normal water-relations was necessary before any possibly abnormalities could be studied (see section VII).

Finally, there is possibility of disturbances in the excretory system, especially in M. sowerbii which lives for many months devoid of any kidney function. This is referred to later in the Discussion.

VII. THE WATER RELATIONS OF TERRESTRIAL MOLLUSCS

(a) Introduction

During an investigation into aestivation and hibernation in snails, Howes and Wells (1934) observed that the active animals underwent continual fluctuations of weight, which, it was claimed, represented periodic changes in the degree of hydration of the tissues. The work was later repeated on slugs (A.ater L. and Limax flavus L.), and similar results were obtained. The slugs exhibited daily weight fluctuations when provided with both food and water, and smaller fluctuations when provided only with water. Animals provided with food but no water rapidly lost weight and died.

The slugs were maintained under laboratory conditions in which the temperature varied 15° - 19° C. and the humidity of the air 40 - 80% R.H. The authors claimed that their results showed that slugs must imbibe free water to counteract the loss by evaporation through the skin.

The present investigation was conducted on the lines indicated by Howes and Wells (1934), and somewhat enlarged. It is considered however to be only an introduction to what is obviously a most complex problem.

(b) Materials and Methods

Much of the present work has been carried out on the species Arion ater L., Agriolimax reticulatus Müller, and Milax sowerbii Férussac. Other species employed were

Arion hortensis Férussac and Limax maximus L.

The vivaria employed were similar to those described by Howes and Wells (1934), consisting of cylinders of perforated zinc, 4 inches in height and diameter, standing on one glass-plate and closed above by another. Food (lettuce leaf) and water when provided, were changed daily. Animals were maintained singly.

For constant humidity work the animals were kept in large, securely corked, tubes, and rested on a perforated zinc platform below which was a quantity of water or water-sulphuric acid mixture giving a known relative humidity.

Anaesthesia was induced by two methods. One was by enclosing slugs in a 250 ml. flask filled with gaseous carbon dioxide; the animals were relaxed after 15 mins. The other method was by the injection into the foot or mantle cavity of approx. 0.6 cc. of a 5% solution of "Myanesin" (α : β-Dihydroxy-γ-(2-methylphenoxy)- propane).

Where decalcification was necessary, the animals were stored in Bouin's Picro Formol Mixture for 4 - 6 weeks, and then embedded in ester wax for sectioning.

General histological structures of the skin were best seen by staining with modified Mallory's Stain (appendix II). The mucus glands were studied from sections stained with Mayer's Mucihaematein (appendix II).

(c) Weight Fluctuations

Fig. 28 shows the weight record of a specimen of A.ater maintained under Howes and Wells' conditions; the temperature varied 15°-20° C. and the humidity 40-80% R.H. Food and water were supplied daily. In Fig. 28 the

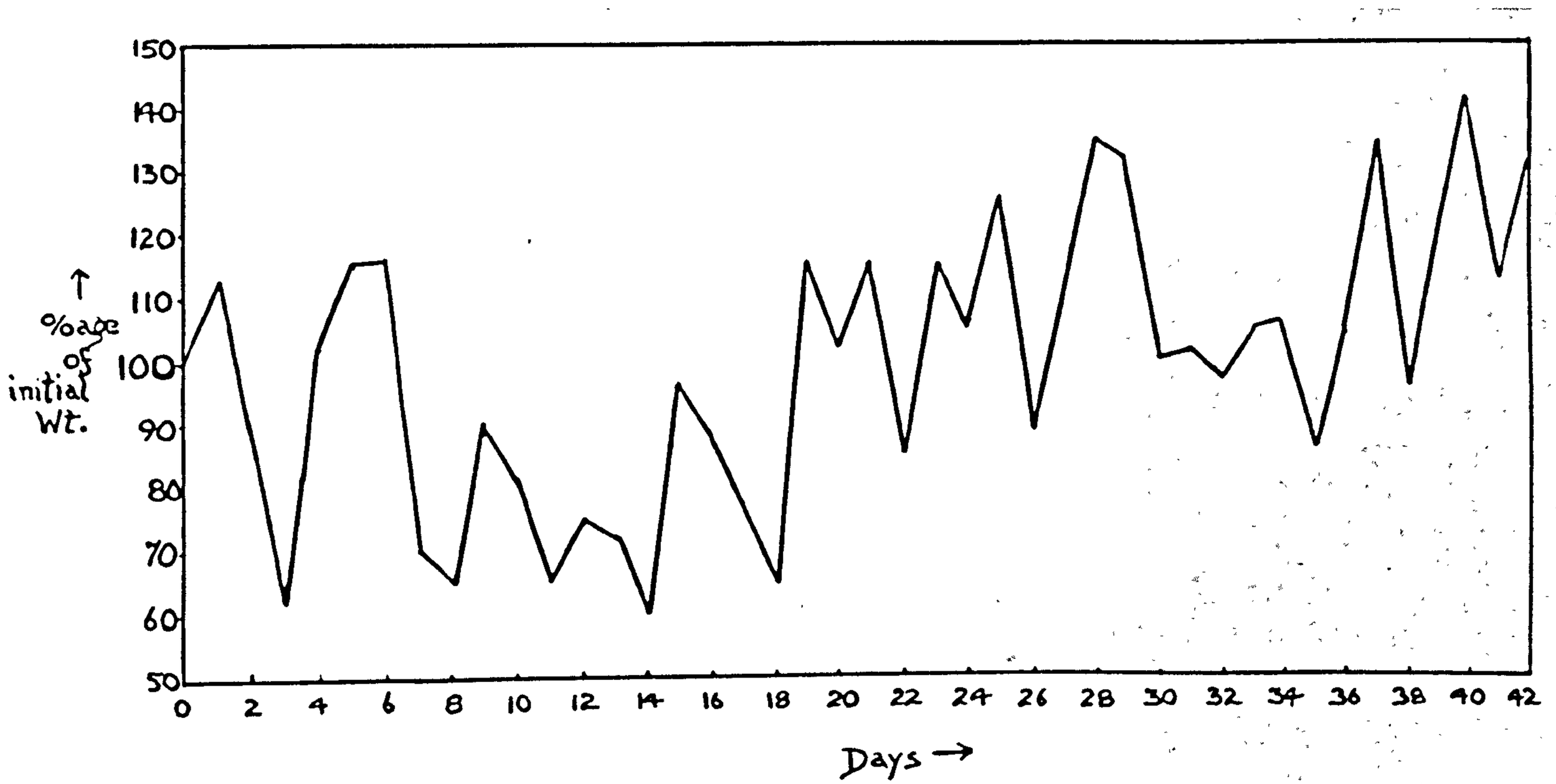


Fig. 28

A.ater: Daily Weight Fluctuations; Food and Water provided (1)

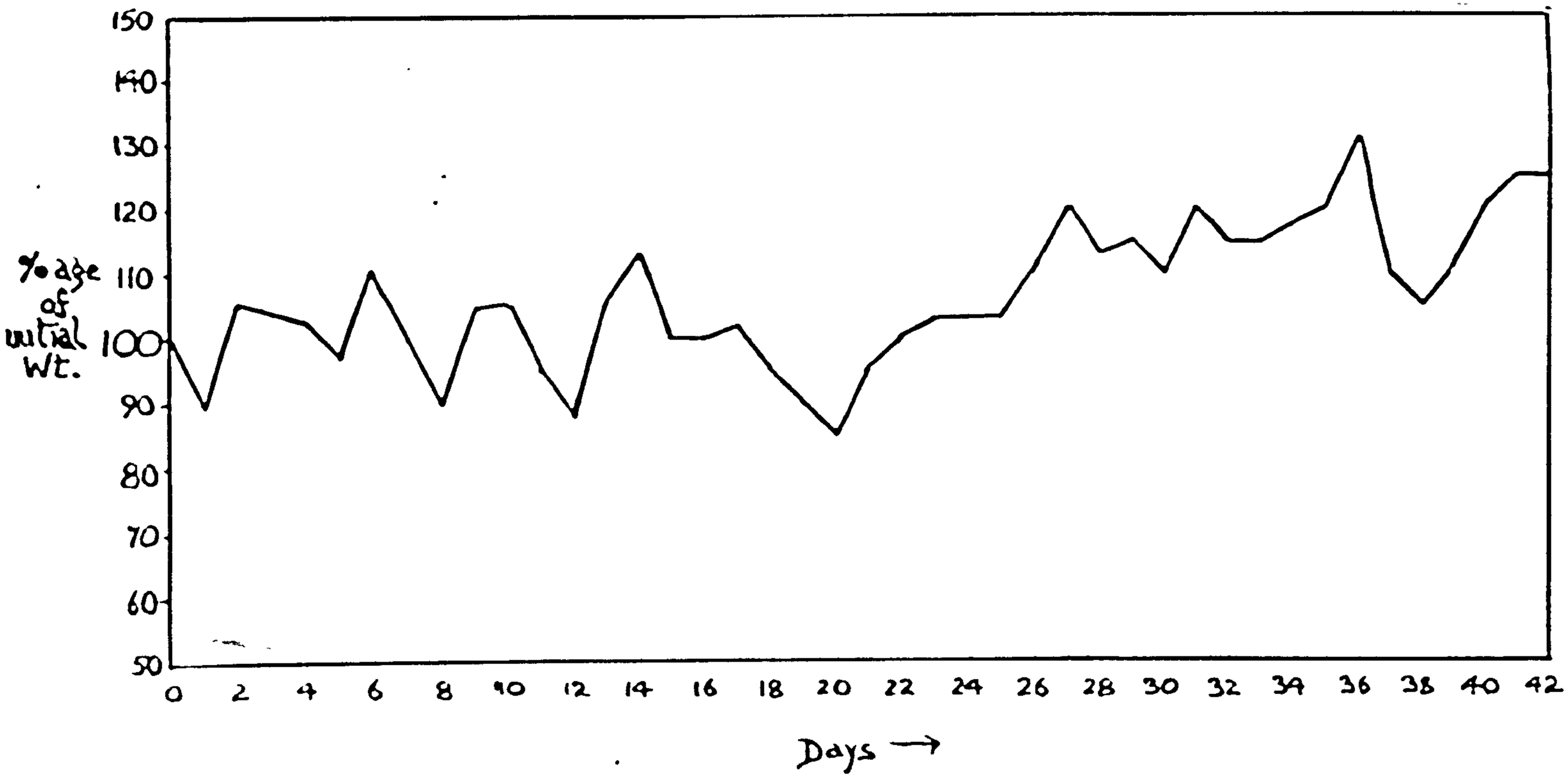


Fig. 29.

A.ater: Daily Weight Fluctuations: Food and Water provided (2)

initial weight of the slug is taken as 100 and it is seen that the weight was not constant, but fluctuated as much as 50% in 48 hours. The cycles of fluctuation did not appear to be of a regular nature, and the normal growth rate of the animal is superimposed on the daily weight graph. Such results, confirming those of the previous authors, were also obtained with M.sowerbii, A.reticulatus, L.maximus and A.hortensis. Fluctuations in A.reticulatus were less than in other species.

Fig. 29 shows the daily weight fluctuations in A.ater under slightly different conditions to those described previously. In changing the drinking-water supply each day, the lower glass plate was not wiped dry, but water was allowed to remain on it. Under these conditions weight fluctuations were reduced, and periods of almost constancy were observed (Fig. 29).

The operative feature is felt to be the availability of the drinking water, as slugs maintain their weight only by the ingestion of free water into the crop (see later). Under the conditions which gave Howes and Wells' results and those of Fig. 28, the water was confined to a small dish; to drink, the animals had to contact the dish and climb the side. As the slugs were inactive during much of each day, water was contacted only very occasionally and long periods were passed without any drinking. In room humidity, evaporation was high, and the water lost was replaced only during the slug's

occasional wanderings up the sides of the dish. This led to sudden fluctuations in weight.

Under the conditions of Fig. 29 however, water was also present on the floor of the vivarium, and was thus more readily available; small movements often brought the slugs into contact with water, and climbing up the dish was unnecessary. Drinking was a more continuous process than before, and the animals' weight was more stable (Fig. 29).

Slugs maintained at 15° C. and 80% R.H. showed similar weight fluctuations to others at 20°C. and 55% R.H. if water was available on the vivaria-floors. If the water was confined to the dishes, then the animals in the lower humidity showed greater weight variations because of the higher rate of evaporation and the fewer occasions on which drinking occurred.

When transferred from 70% to 90% R.H. where drinking water was only in the dish, slugs often lost weight, but usually regained it when transferred from 90% to 55% R.H. if water was available on the floor.

The presence of free-water in the vivaria did not affect the relative humidity of the air.

It is concluded that slugs undergo certain fluctuations in weight under laboratory conditions, but that these fluctuations have been exaggerated by previous authors because of the particular conditions operating within their vivaria.

(d) The Need for Free Water

Slugs were maintained as described except that free water was not provided. The results are given in Fig.30.

The animals lost weight catastrophically, and survived for only 3 - 4 days. This confirms the view of Howes and Wells (1934) that the principal factor controlling the hydration of the animals is the imbibing of free water into the crop.

Water-loss from slugs under unstimulated conditions is principally by evaporation from the skin. Other contributory causes are excretion, respiration, slime-secretion, and other vital processes. During continuous movement, much water may be lost in mucus-secretion, but under normal conditions these vital processes appear to be responsible for little of the total loss. A typical "drying-out" curve was virtually one straight line which continued through the death-point until the final dry-weight was reached, regardless of whether the animal was alive or dead. The water lost in this manner must be replaced by ingestion of other water into the crop.

A number of A.ater were suspended in such a way that the body region from the posterior extremity to the posterior edge of the mantle was immersed in water, leaving only the mantle and head exposed to the air. A collar of thin cardboard fastened round the mantle region prevented the head being turned downwards and water being drawn into the crop. Under these conditions slugs lost water through those parts of the body exposed to the air, and being

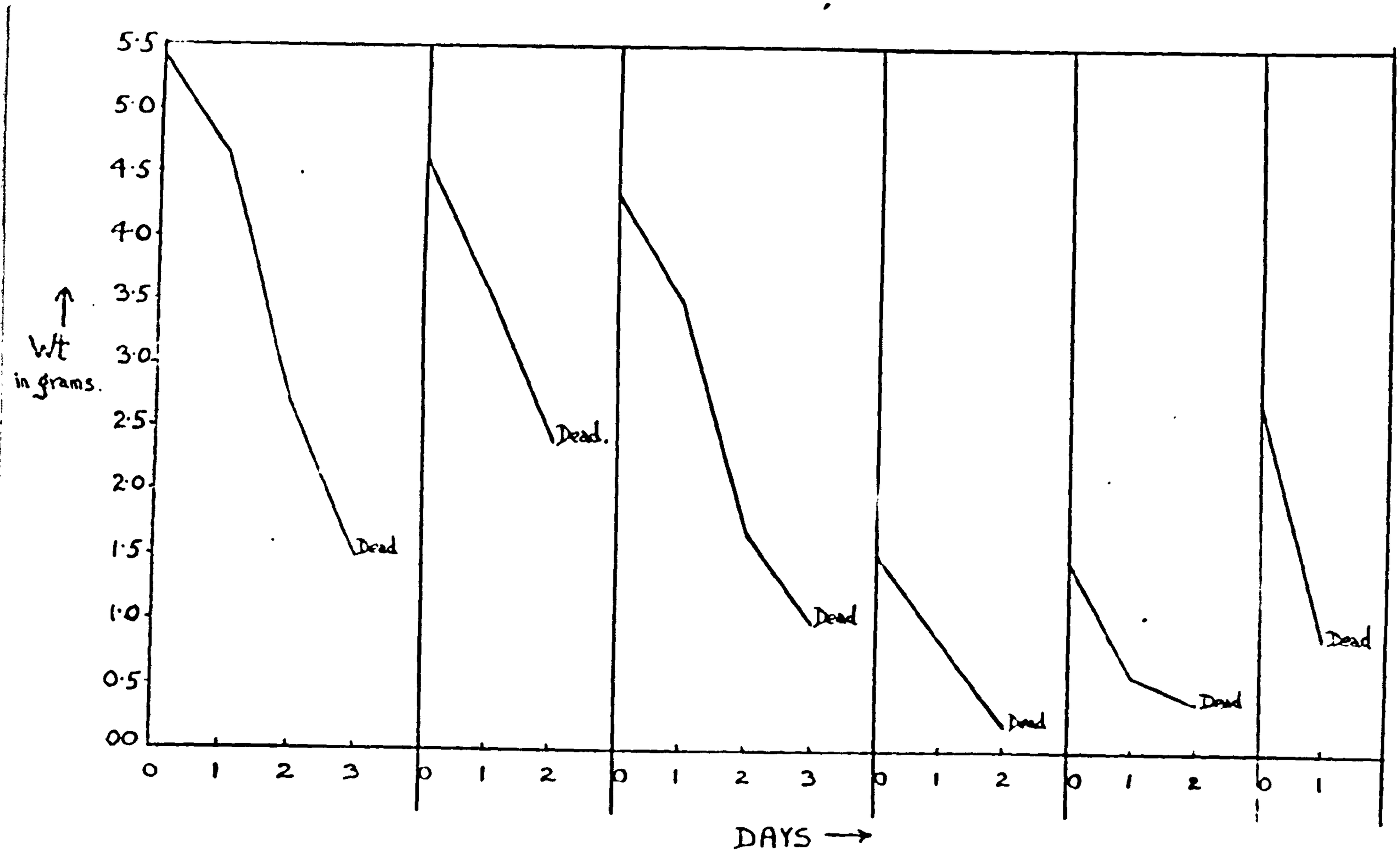


Fig. 30.

A.ater: Weight Records; Food only provided.

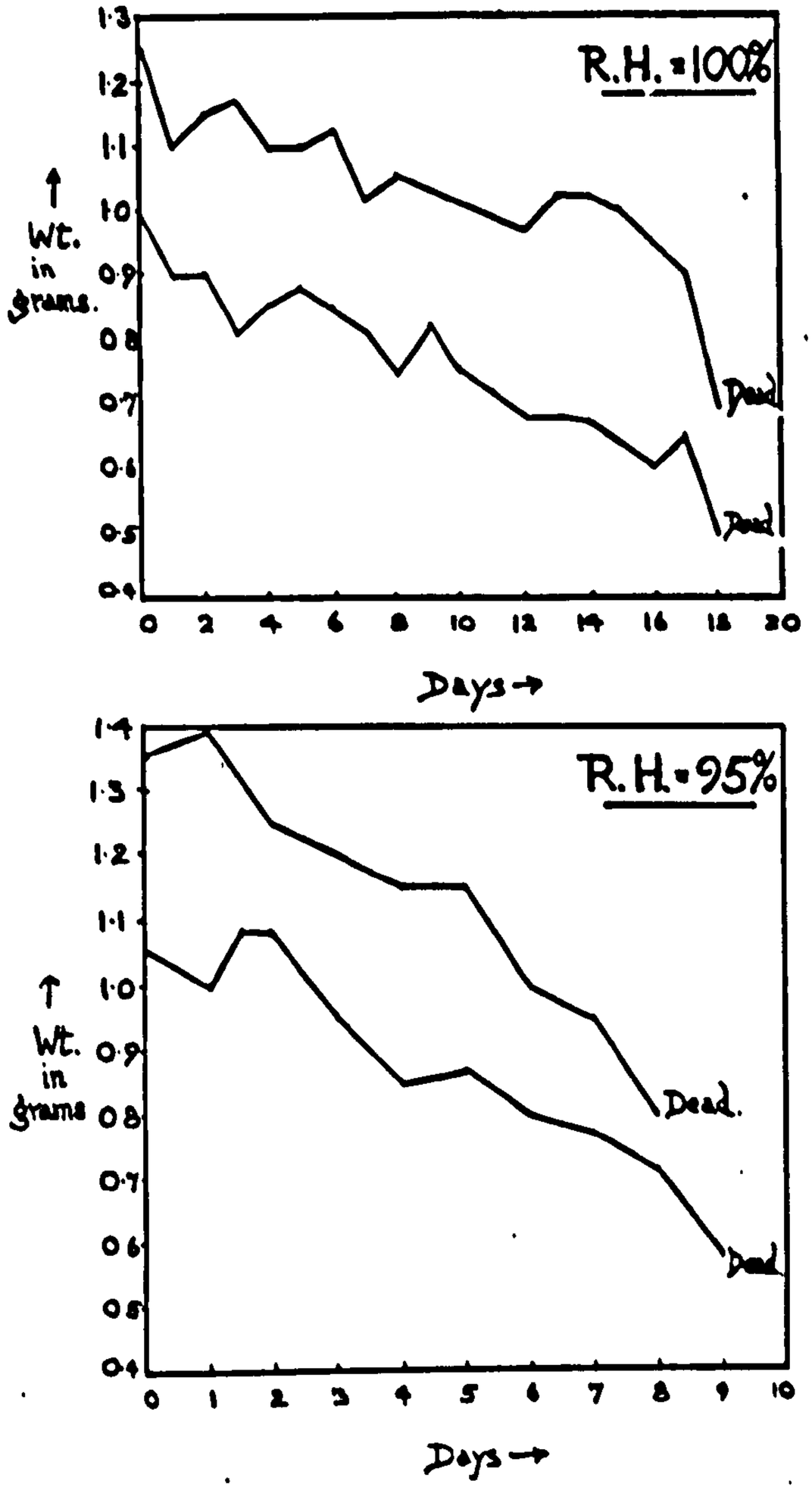
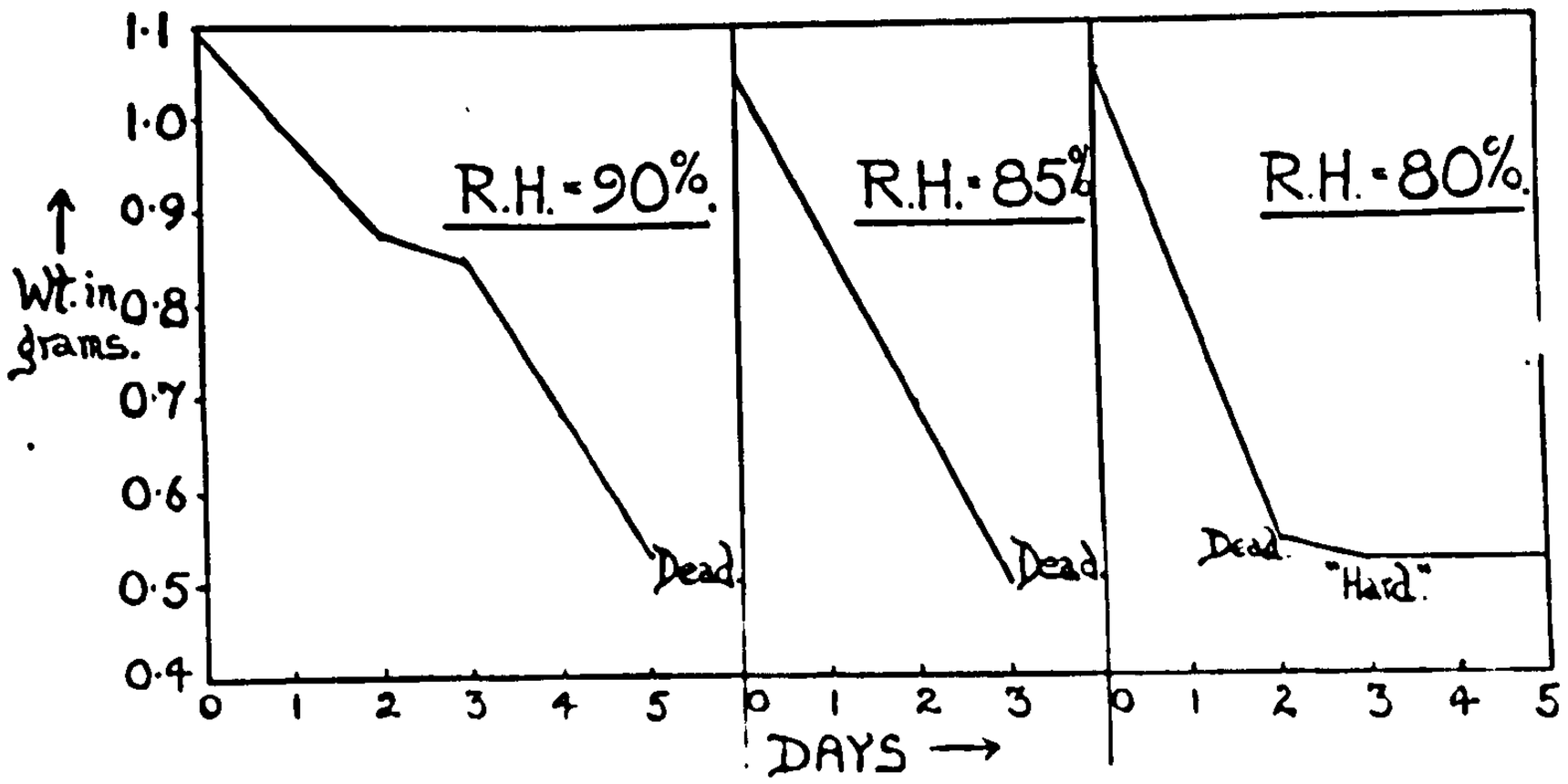


Fig. 31

A.ater: Reactions t Constant Relative Humidity.



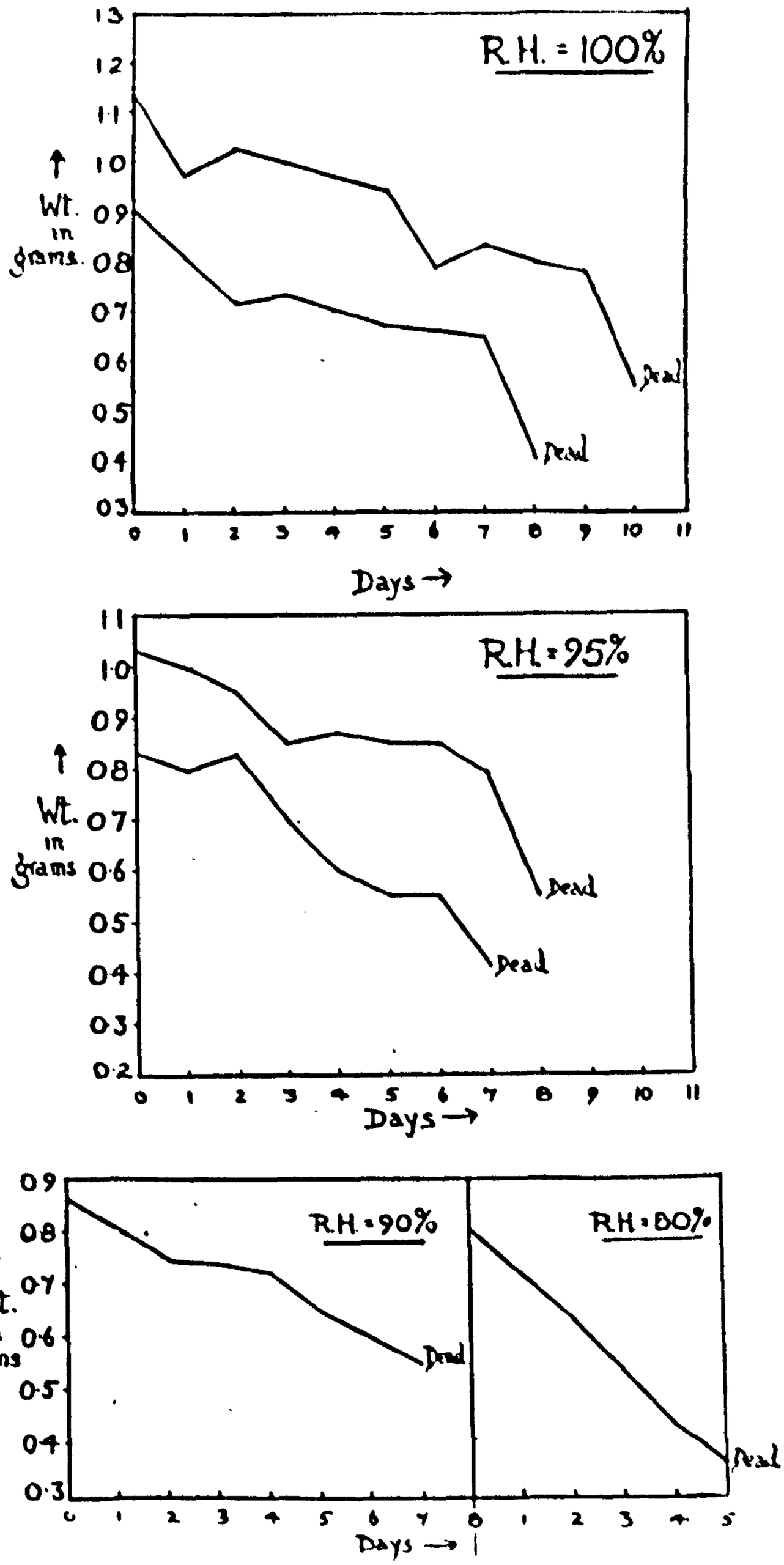


Fig. 32

A. reticulatus: Reactions to Constant Relative Humidity

unable to drink, lost weight and died within 2 days. Control animals under similar conditions but which were supplied with drinking-water did not lose weight, and their deaths after 4 - 5 days were probably due to the physical injuries sustained in the obtaining of the experimental conditions. The animals were apparently unable to absorb water through the skin which was immersed in water. Other experiments (pp.131 and 134) have given similar indications.

Investigations were made to see if slugs could survive in the absence of free-water in atmospheres of high humidity.

Animals were maintained in tubes (p.115) at known humidities. The presence of food in the tubes did not affect the humidity. The results are given in Figs. 31 and 32.

Fig. 31 shows that A.ater lost weight under such conditions and finally died. At humidities below 95% R.H. the loss was rapid and the animals died within a few days. At 95% R.H. the loss was reduced, death occurring after about 10 days. At 100% R.H. the animals survived for a considerable time without free-water. The average length of life was 2 - 3 weeks, one individual surviving for 38 days. In all cases, lack of food supply (dry) reduced the survival period by about half.

Fig. 32 shows the reactions of A.reticulatus to similar conditions. Compared with A.ater, the actual humidity appeared to be less important than the absence of drinking-water. At 80% R.H. the slugs lived longer (average 4 - 5 days) than did A.ater; similarly at 90% R.H. (average 7 - 8 days). At 100% R.H. the species survived less well than A.ater (average 8 - 9 days, longest recorded 17 days). Food (dried lettuce-leaf) was again important to survival.

There appears then to be specific differences in the tolerance of these conditions by slugs. This will be referred to later.

None, however, survived the absence of free water, and even in 100% R.H. lost weight due to evaporation. This rendered the surrounding atmosphere super-saturated and led to condensation on the sides of the tubes. Although wiped daily, some of this water may have been ingested by the slugs, but not in sufficient quantity to sustain their weights.

In areas where the population-density of L.maximus L. is sufficiently high for observations to be made, it has been observed that the species appears to be active under drier conditions than others. The observations already described were therefore repeated on L.maximus, and the results are shown in Figs. 33 and 34. Fig. 33 shows the normal daily weight-fluctuations, with water present on the vivarium floor. The irregular weight rhythm observed in other species was again evident.

Fig. 34 A and B shows that removal of the water supply again led to a catastrophic dehydration and death after two days. Fig. 34 C gives the weight records of L.maximus at 100% R.H. without drinking-water. The average period of survival under these conditions was about 3 days, considerably less than for other species (p.124-5). Thus, although possibly active in drier field conditions, this species in the laboratory appears to have even narrower limits of tolerance than do others.

(e) The Reactions of Small Slugs

Howes and Wells (1934) used animals of an initial weight of approximately 5.0 - 7.0 gms. In repeating the work, some very small individuals of A.ater and M.sowerbii ($<$ 0.6 gms.) were used, and found to give contrasting results.

Fig. 35 shows that these small individuals still gave typical drying-out curves even when supplied with food and drinking-water. Although these results may be due to the conditions in the vivaria, their possible significance is discussed on p.156 .

(f) A Possible Mechanism

As weight fluctuations have been definitely established (P. 119), even in the absence of drinking-water (Figs.31-32), it is suggested that there may be some

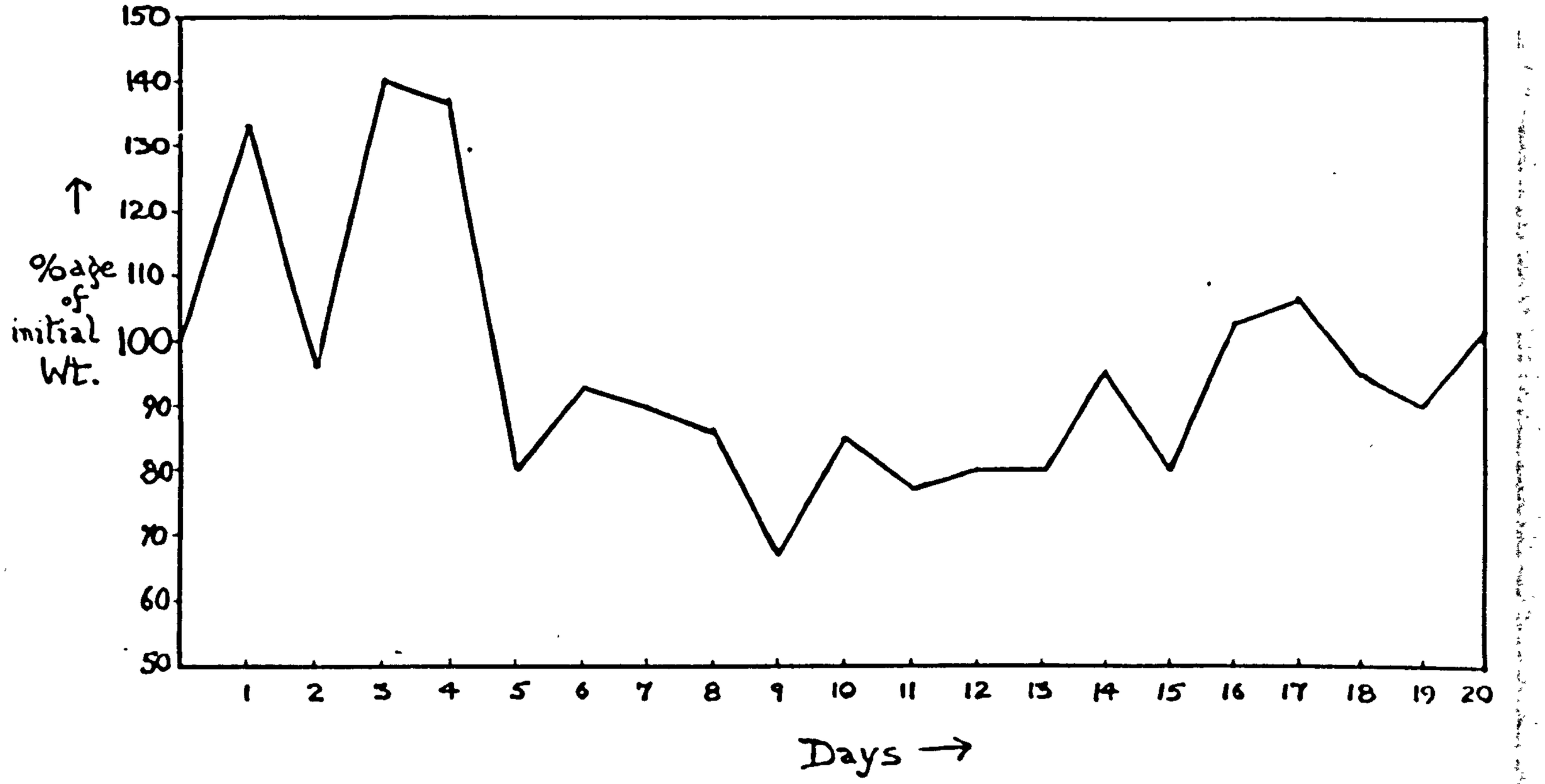
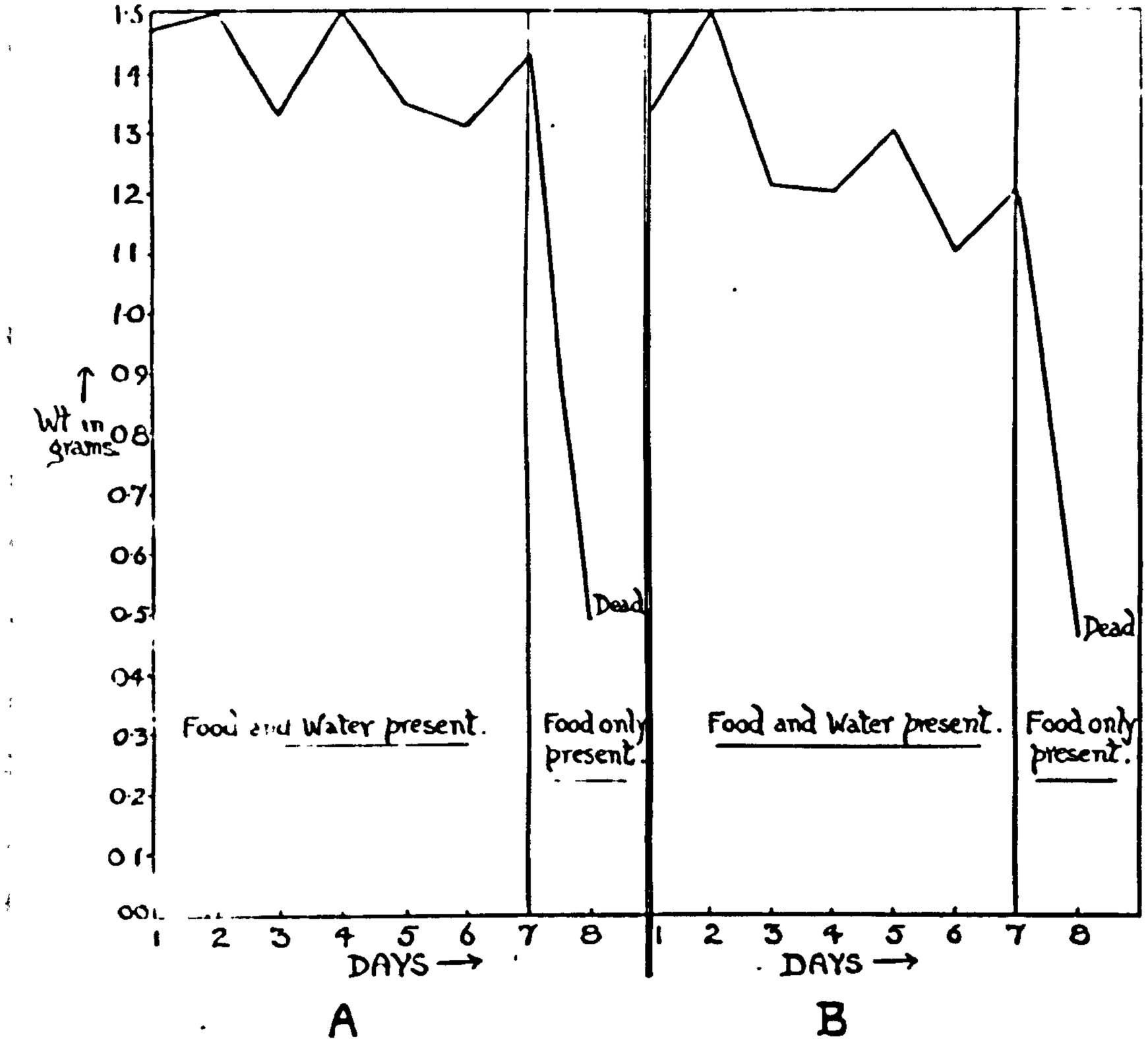


Fig. 33.

L.maximus: Daily Weight Fluctuations: Food and Water provided.



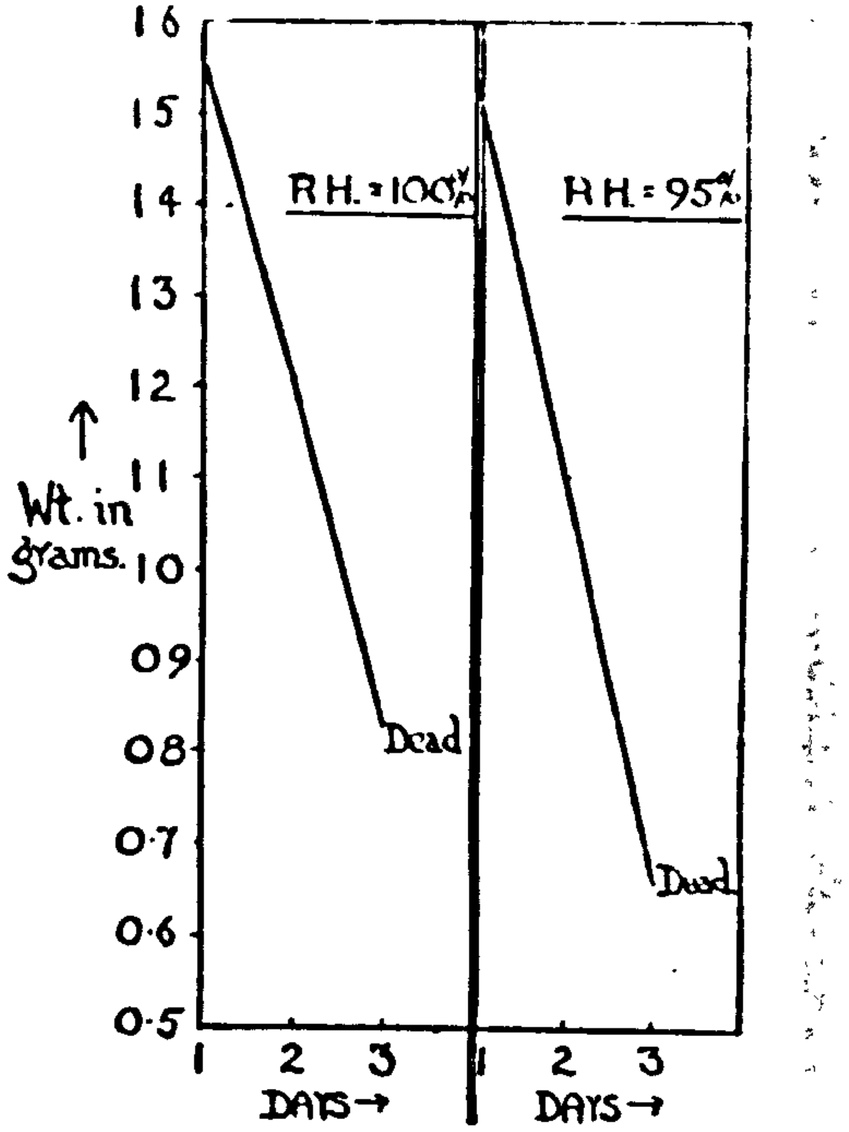


Fig. 34

L. maximus: A, B, Reaction to deprivation of Free Water
C, Reaction to Constant Relative Humidity.

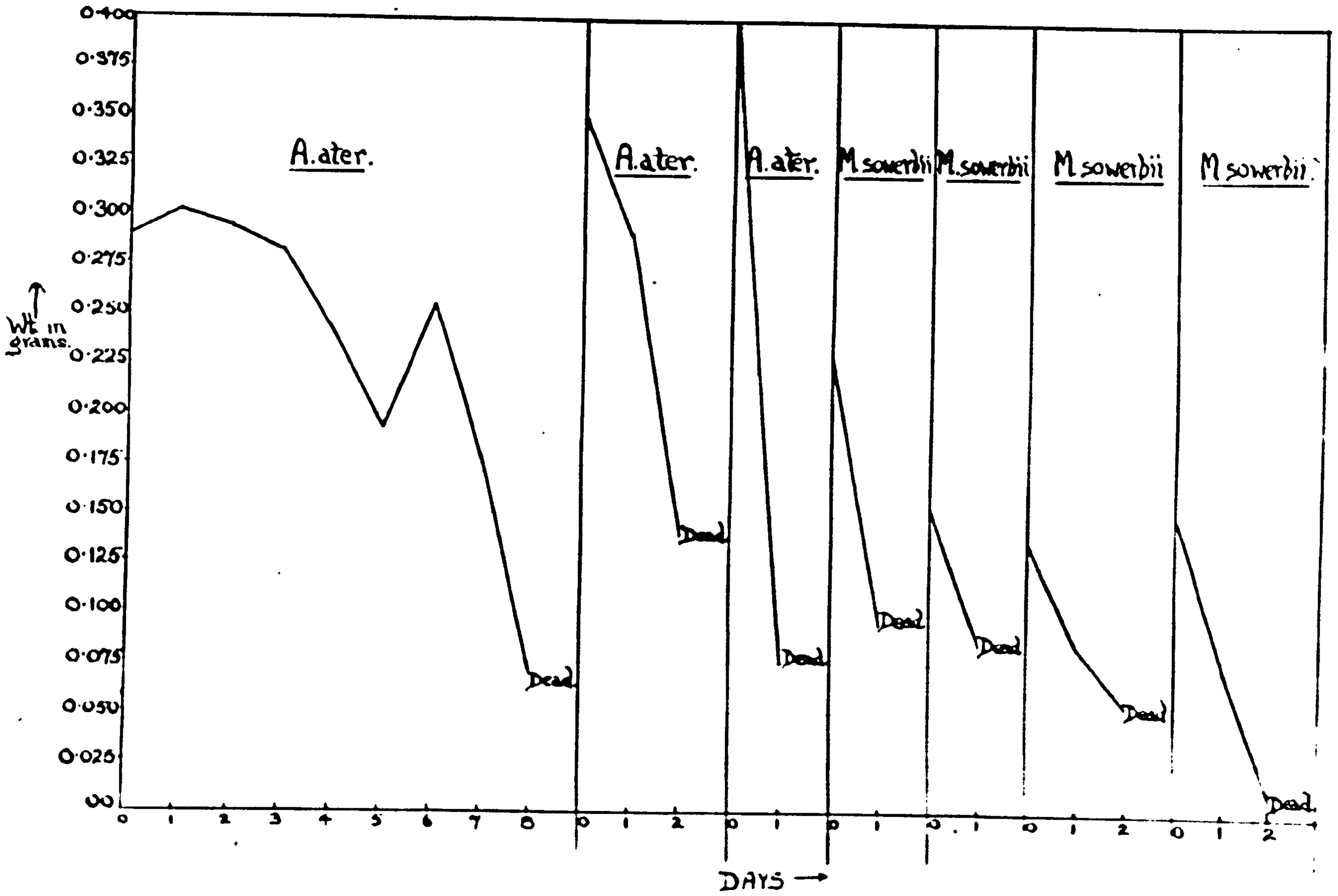


Fig. 35

Weight Records of Small Slugs: Food and Water provided.

periodic physiological mechanism for the conservation of water in slugs. This mechanism may act during the upward stretches of the normal weight record, conserving the water drawn into the crop. Possibly owing to the magnitude of the task it tires after a time and ceases to function, allowing water to be lost by evaporation (the downward stretches ofthe record). Water is again conserved when the mechanism is rejuvenated.

Such a mechanism could be correlated with the metabolic rate of the animal. The reduction of this rate to a low, constant, level by anaesthesia (p.115) was attempted, and the effect on the hydration cycles noted. The results were not significant as the animals lost weight steadily and died after 2 - 3 days probably because of their inability to imbibe free water. Oral injection of water under these conditions failed to prevent death.

Slugs maintained at 100% R.H. without drinking water lived for a time, showing slight fluctuations ofweight superimposed on a steady decline. It is probable that the rate of water-loss was in some way proportional to the R.H. of the surrounding air; temperature changes during exposure to constant R.H. had no observable effect, so saturation deficit was apparently not important. At 100% R.H. the mechanism may be able to control the water-content of the animal for a time owing to the reduced rate of evaporation. Under these conditions, water loss in the 24 - 48 hours preceeding death was often suddenly accelerated. This may have represented the breakdown of the conserving system, and shown the true rate of loss.

(g) Reactions to Changing Humidities

Observations were made on A.ater to determine the animals' reactions to changing humidities in the absence of drinking water, and to test their power of recovery from rapid dessication under these conditions.

The animals were exposed to a low R.H. for a number of hours, and then transferred to a saturated atmosphere, their weights being taken at intervals.

The results are shown in Fig. 36. During their exposure to a low humidity, the animals lost weight rapidly due to evaporation. When transferred into saturated air, they failed to take up water through the skin, or even to hold the evaporation in check. Fig. 36 A and C showed a greater loss during the first hour in 100% R.H. than in the previous lower humidities; B and D showed a loss about equal to the previous one. The actual R.H. to which they were exposed did not apparently affect their reactions on returning to saturated air. In all cases water continued to be lost, and death occurred about 48 hours after the beginning of the experiment.

In Fig. 36 B and D drinking-water was provided for the last 15 hours of the experiments. It failed to save the animals because dessication had so inhibited locomotion that they were unable to approach and imbibe

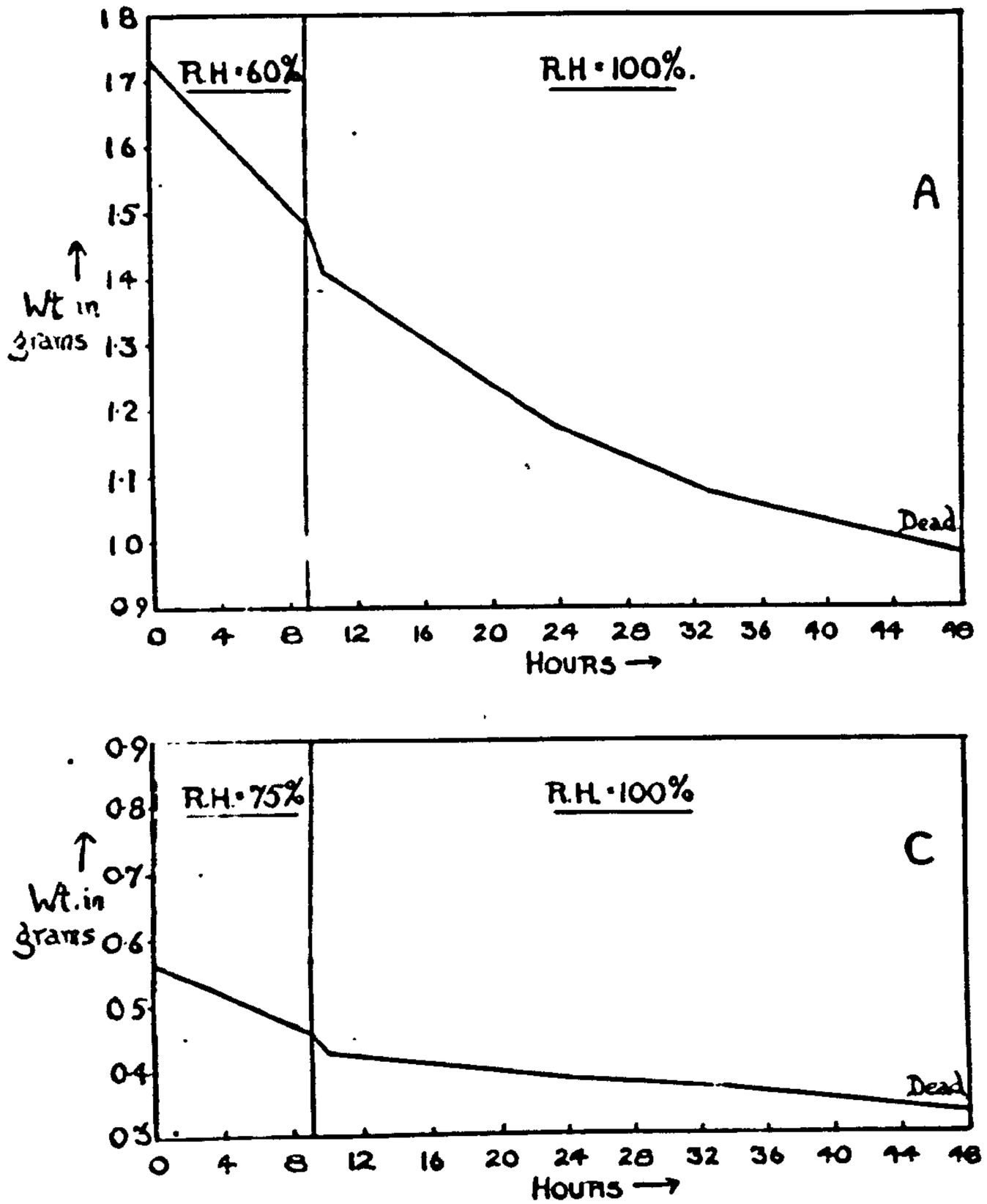


Fig. 36

A.ater: Reaction to Changing Relative Humidity

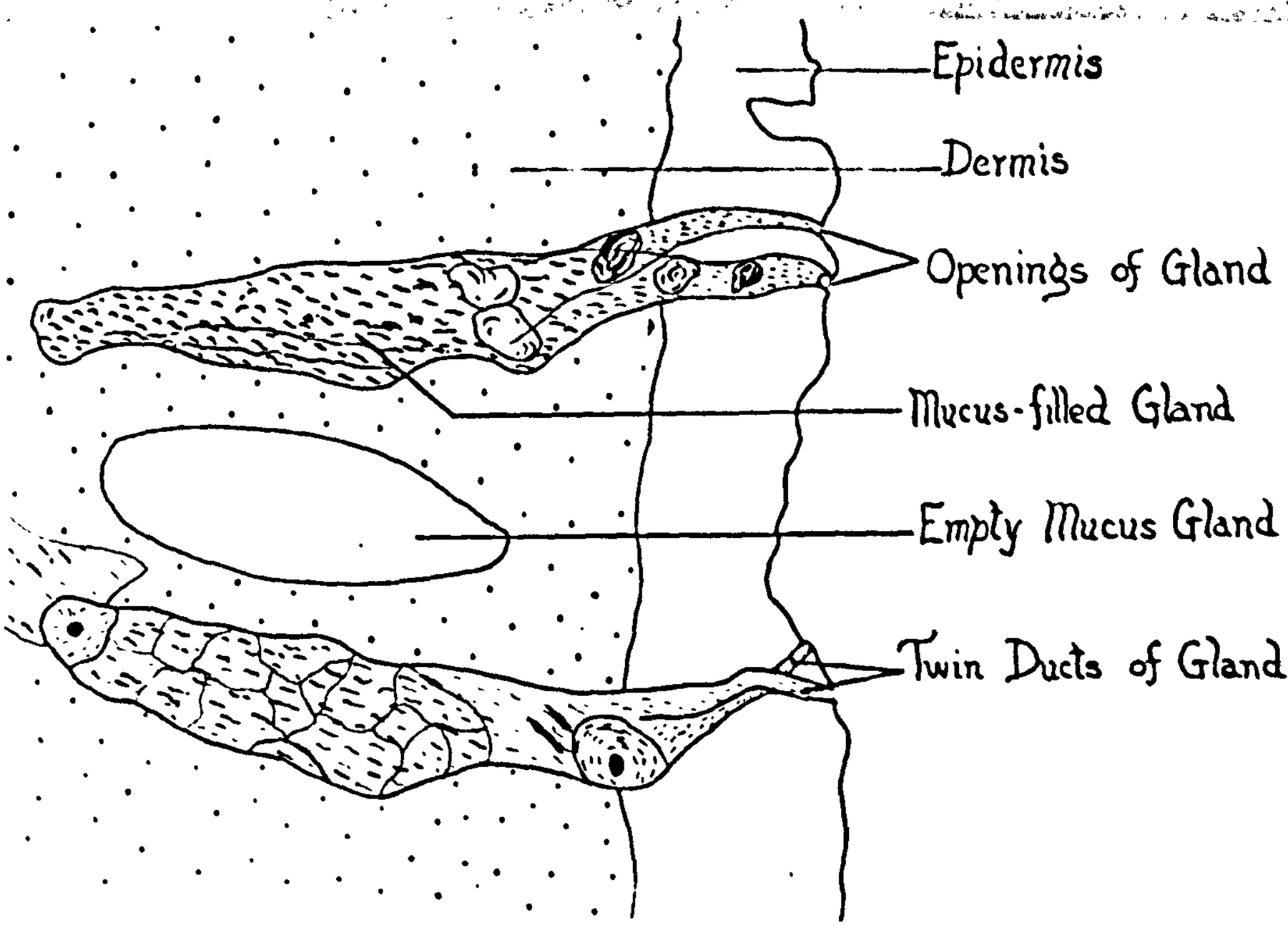


Fig. 37.

M.sowerbii: Mucus Glands.

the water. Control animals, similarly dessicated, were orally injected with about 0.1 ml. per hour of water by an "Agla" microinjection syringe as described by Cragg and Vincent (1952). These animals showed a 70% recovery rate from dessication.

It appears then that if slugs are exposed to atmospheric dessication of any appreciable extent, a return to saturated air does not save them unless drinking-water is present. And further, that even this is useless if dessication has impaired the animals' locomotion.

Fig. 31 showed that A.ater could survive for 2 - 3 weeks in 100% R.H. without drinking-water, and that their weight decreased considerably before death occurred. Fig. 36 shows that if previously dessicated for a short time, even if the actual weight loss is small, the animals' resistance to 100% R.H. without drinking-water is only 24 - 48 hours. It seems possible that the operative factor is not the extent of dessication as much as the speed of dessication.

(h) The Mucus Glands

The skin of A.ater, M.sowerbii and L. maximus was examined to determine the form and distribution of the mucus-glands.

The glands differed both in shape and size, varying from long, slender structures, to others of spherical

shape. Typical glands of M.sowerbii are shown in Fig. 37.

The glands appeared to be intracellular in the form of modified dermal cells. The cell-wall was thin and lined with an extremely thin layer of protoplasm. The remainder of the cell was occupied by a large vacuole filled with mucus. The origin of the mucus is unknown but is is possibly of nuclear origin. Nuclei were not observed. From the outer end of the gland arose two distinct ducts, which crossed each other and opened on the body surface by two separate apertures. The main body of the gland lay in the dermal tissues, the ducts traversing the epidermis. Rounded, solid, objects of unknown affinity were seen in both glands and ducts.

When empty, the glands appeared as clear, open spaces. No specific differences were observed, although those of L.maximus were sometimes more slender and well-defined than in other species.

The distribution of the glands was variable between individuals. Where they occurred, they did so indiscriminately over both ridges and hollows of the skin.

At the posterior of M.sowerbii the glands were virtually absent from the foot, except at the lateral margins. Those of the sole were very few and small. The lateral and dorsal surfaces of this region however were liberally supplied.

In the median region of M.sowerbii posterior to the

mantle, again only a few small glands occurred in the foot. Further, many individuals were devoid of glands on the dorsal surface of this region. On the lateral surfaces, especially the ventral-lateral, a number of small glands occurred, becoming more numerous in the region approaching the posterior edge of the mantle. In L. maximus the number of glands on the lateral surfaces of this region was greater, and some also occurred on the dorsal surface.

In the mantle region of M.sowerbii, no glands were observed in the foot of many individuals, and very few on the dorsal surface, although some specimens had a fair profusion in the latter locality. In all cases a number were observed at the lateral margins of the foot and on the lateral body surfaces. Some also occurred in the roof of the mantle-cavity, and a smaller number in the floor of the cavity.

Slugs were killed and fixed within a few seconds of hatching. No difference was observed in the form or distribution of the glands of these specimens.

The foregoing description is a general pattern from which many individuals differed in detail.

No specific differences were observed in the distribution.

VIII DISCUSSION

The first reference to the genus now known as Brachylaemus was by the Frenchman Dujardin in 1843, resulting from his discovery of a new trematode parasite in the shrew, Sorex leucodon Zimmermann. Within his new genus Brachylaima he included several other distomes, most of which have since been transferred to other genera. Unfortunately, Dujardin's descriptions of his animals were poor, although probably adequate to distinguish them from other species known at the time. As new forms were found however, Dujardin's descriptions, including that of his type-species B.advena, came to apply equally to a number of obviously distinct groups. But not until 1928 was the position clarified by Baer's re-examination of the genus and statement of characters. This involved abandoning the original type-species B.advena, and its replacement by B.migrans, as the first species to be adequately described.

Synonymy was introduced into the group by the erection of the genera Harmostomum Braun 1899 and Heterelope Looss 1899, the subfamilies Harmostominae Braun 1899 and Heterolopinae Looss 1899, and the family Harmostomidae Odhner 1912.

Joyeux and Foley (1930) resolved the synonymy, establishing the correct names for family, subfamily, and

genus as Brachylaemidae, Brachylaeminae, Brachylaemus respectively. Recently some authors have reverted to the original name Brachylaima for the genus, while the names based on Harmostomum are still encountered. The present author continues to use the generic name Brachylaemus, as the commonest in modern usage.

The classification of the brachylaemids has been attempted by many workers, many schemes being based on the same observed characters. Failure to agree on the relative significance of these characters has led to a succession of attempts, none of which is universally accepted. That of Allison (1943) is widely acknowledged but possibly only as it is the most recent one.

Allison pointed out the need for further studies on life-histories and the development of the genital and excretory systems. This seems very wise, as much of the present confliction is felt to be due to studying parasites only as stained preparations. Under such conditions many look very different to their appearance "in vivo", and such organs as the excretory system become indistinguishable. Description of living specimens eliminates the possibility of including the effects of fixation as "anatomical characters", and usually gives a fuller picture of the internal anatomy.

The present species possesses a caudal appendage during early metacercarial life and, it is believed, during the cercarial stage. The single sporocyst found in 1949 in

a specimen of A.ater contained cercariae indistinguishable from young brachylaemid metacercariae. If, as seems likely, this sporocyst was of the same species, a number of observations can be made regarding the life-history. The cercariae are produced directly from the sporocyst tissues, and there is no redial generation; the incidence of sporocyst infection is low, but each sporocyst produces a large number of cercariae; and the sporocyst and cercarial phases are produced in a different species of host to that which harbours the metacercariae. Confirming the last point, no host has been found to harbour both sporocyst and metacercariae. These points, if true, agree with direct observations on known brachylaemid life-histories. Although there are no previous records of brachylaemid parasitization of Agriolimax reticulatus Müller, various references to infection of Agriolimax agrestis L. are thought to refer to A.reticulatus, owing to the former confusion of these species (Luther 1915; Quick 1949).

The four developmental stages have been named caudal, feeding, corrugated and smooth respectively. The corrugated stage may be unique in brachylaemid development (Dollfus 1954 - personal communication). The cuticle of other metacercariae has been observed to wrinkle with the twistings of the animal, but there is no previous record of a permanently corrugated stage of

some weeks duration. In the present species the cuticular corrugations are maintained regardless of the stretchings of the body. Dawes (1946) inferred the absence of cuticular corrugations to be a characteristic of the family, although this apparently referred only to the adult flukes.

The metacercarial cuticle is thick, averaging 9 μ. During the corrugated stage this figure may reach 15 μ. It seems possible that it may serve as a replacement for the cyst which is usually present around such larvae. It is extremely resistant to penetration by intra-vitam dyes, and is quite flexible. A resistant yet supple cuticle such as this is probably an improvement on a typically rigid cyst, allowing more freedom of movement and possibly requiring less digestion after being eaten by the vertebrate host. Estimations of the thickness of the cuticle in other unencysted metacercariae are not available for comparison.

Dujardin (1845) mentioned several brachylaemid metacercariae which he found in molluscs, noting in some cases that they resembled the adult stages in mammals except that no genital organs were visible. Although rudiments of the reproductive system may have been present but overlooked by the author, the system was obviously not in an evident stage of development. Subsequent descriptions of other metacercariae have also noted this fact, although Ulmer (1951) observed

the beginnings of all adult genital structures in metacercariae of Postharmostomum helicis. In the present species, although the outline of the testes, vasa deferentia, and genital pore have been observed, no trace has been found of any other genital organs. It appears then that in most brachylaemid species, the reproductive system is in only an early stage of development in the metacercariae, and must await ingestion by the vertebrate host to attain full development.

. The excretory system and ciliated ducts of larval trematodes have recently been recognized as important taxonomic characters. As the canals tend to be visible only in part at any one time, the present description has been compounded from a number of observations. In future work, care must be taken that sufficient observations are made, under suitable conditions, to exclude the possibility of unrelated ducts being recorded as synonymous or joined.

Two interesting features emerge from the description given on pp. 36-40 . The first is the occurrence of complete cross-connections joining the right- and left-hand sides of the system. The main anterior transverse tubule (Fig. 6) appeared to join the two ciliated ducts. There are previous records of such connections, but rather unreliable ones. Leidy (1850) referred loosely to "anastomoses" of tubules, but

as he considered the whole system to be a "vascular system" his records cannot be relied upon. Dujardin (1845) had previously referred to cross-connections, but his descriptions are notoriously inadequate. Mehra (1936) claimed that in Harmotrema nicolli the "... two outer trunks of each side are joined by a transverse connection", and that "... the two inner trunks are also connected by a transverse vessel at a little distance in front of the ventral sucker." This latter record appears to be in accordance with the present work, but a final decision must be withheld for more detailed work.

The second point concerns flame-cells. These are considered to be absent in the present species. Previous workers on certain brachylaemids have reported seeing flame-cells in large numbers, and it is felt that if any were present in this species, some would have been observed.

Their absence may be correlated with the presence of ciliated excretary ducts. The continual beating of the cilia in a posterior-anterior direction will force onwards any fluid between the upper limit of the cilia and the excretory pore (Fig. 6). Simultaneously fluid between the lower limit of the cilia and the distal ends of the system will be drawn along owing to the tendency to create a vacuum. Thus all fluid in the system will be continually moved towards the excretory pore, and as there are no extensive, fine, ramifications of the

tubules, this ciliary action may well be sufficient to replace the fundamentally similar action of individual flame-cells.

The only brachylaemid subfamily in which flame-cells have been definitely observed is the Leucochloridiinae; and this group is diagnostically separated from the rest of the family by the absence of ciliated ducts and the presence of finely branched excretory tubules.

The final answer again awaits more extensive work. In determining whether flame-cells and ciliated ducts are alternative mechanisms, care must be taken to ensure that "flickering" within excretory tubules is in fact due to flame-cells or cilia, and not to sperm-tails or the streaming of droplets as in Fasciola hepatica (Stephenson 1947).

It was found that the number of parasites within a host decreased as one developmental stage succeeded another. To propose a migration out of the host is impracticable as it implies a desertion of the host when the larvae are immature, and they can attain maturity by remaining within the host. In no host have any dead metacercariae been found however, and dead parasites are known to remain recognizable despite cytolysis when maintained in kidney tissue for 2 - 3 days after death. Any larger scale death-rate within the host then appears unlikely.

A possible explanation is connected with the lethal effect on the host. Many harbour considerably fewer parasites of any one stage than the average, and these may survive to harbour the following stage. The hosts harbouring the maximum number of larvae may succumb to their effects, and thus the only hosts included in future collections will be those with the least numbers of parasites.

The caudal stage larvae may exert no physiological effects on the host, but where occurring in great numbers (100 - 150) could cause death by pressure on the heart, which is surrounded by kidney-tissue. This is a known cause of death in A.reticulatus infected by Rhabditis sp. (Nematoda).

Although an adult fluke has not been observed, a life-history for the species has been proposed on the basis of the known features of the infection. All known Brachylaeminae use either birds or mammals as definitive hosts. Although Song-Thrushes have been seen to eat M.sowerbii in the infected area at Durham, it is thought that a mammal is the most likely vertebrate host. If a bird were involved, a wider and more even distribution of the parasite would be expected, even allowing for "territory".

Small mammals, whose period of activity is coincident with that of the intermediate hosts, have been

recorded as hosts for brachylaemid species by previous authors. Some (Stiles and Stanley 1932) have further recorded slug species as the secondary hosts involved. It is suggested then that a small mammal (hedgehog, mouse, vole, etc.) ingests smooth stage metacercariae in slugs during the autumn. The adult flukes come to maturity during the winter, and viable eggs are produced during the spring, passing out with the faeces. A sporocyst and cercariae are produced. The latter are indistinguishable from caudal stage metacercariae, but it is thought that a transfer to a second intermediate host occurs between the two stages. This may or may not be of the same species as the first host, but no host harbouring metacercariae also contained as sporocyst, and the only sporocyst found was in A.ater, which does not normally harbour metacercariae. Baer (1952) records that brachylaemid cercariae may re-enter the first host via the urinary pore, as well as penetrating other individuals of the same species in the same way. This could happen very easily during coitus. Penetration into another slug species would also be feasible via the ureter when the slugs are in contact during normal wanderings.

The larvae, now termed caudal stage metacercariae, thus appear in the second intermediate host in March-April, and come to "maturity" in the autumn, when ingestion by a vertebrate starts another cycle.

The fact that those metacercariae appearing first in the slugs reach maturity early enough to allow a vertebrate infection to be established and viable eggs passed out in the late autumn may itself be a pointer to a likely vertebrate host. From the appearance of this secondary autumnal infection, it is evident that only a short time elapses between the vertebrate's ingestion of mature larvae and the production of eggs from the adult fluke. Any suitable vertebrate ingesting larvae during the autumn will carry mature adult flukes throughout the winter. The rodents are not true hibernators, and during a spell of warm weather in the winter will become active for food. In such sorties, trematode eggs, if present, will be passed in the faeces, leading to occasional instances oflarval infection among the slugs in the Dec. - Feb. period. The fact that no such cases are known suggests that the vertebrate host is a true hibernator, passing no eggs until the resumption of full activity in the spring.

The failure to infect hedgehogs artificially with the brachylaemid may have been due to their being "wild" specimens already carrying heavy nematode infections. This may have provided an immunity to further infection, even by trematodes. Guinea-pigs, mice, and chickens also proved refractory, and this brachylaemid species appears to be fairly specific. Several previously

described species have been quite unspecific. B.virginiana inhabited opossum, dog, cat, rat, and chicken (Krull 1943); B.suis was reared in pig, rabbit, rat, mouse, turkey, and pigeon, although guinea-pig and chicken were refractory (Balozet 1936, 1937). Some brachylaemids then appear to be less specific than the present one. Previously described species have also been found to inhabit a variety of molluscan hosts; the present species has been found only in M.sowerbii and A.reticulatus (except for a single case in A.ater), although at least 10 other molluscan species are common in the infected locality.

As they are of little economic interest, the effects of larval trematodes on their hosts have not been extensively studied in the past. Faust (1920), Agersborg (1924), Hurst (1927), Wesenberg-Lund (1931, 1934), Rees (1936) and Rothschild A. & M. (1936, 1939, 1941) however concluded that the cercarial and sporocyst stages damage their hosts and often lead to death. Wesenberg-Lund (1934) and Cort, Olivier and McMullen (1941) emphasised the importance of larval trematodes as decisive factors in controlling host populations. Although only one sporocyst has been observed during the present investigation (Cragg and Vincent, unpublished) a number of its effects on the host were briefly noted. There was a stimulation of the shell-gland, and an abnormal deposition of granules of calcium carbonate. Further, the main centre of sporocyst activity, namely the albumen gland and oviduct, had become extremely necrotic in

appearance. Further investigations were obviously impossible.

The present investigation of the effects of the metacercariae on the hosts is apparently without predecessor in the literature. Previous workers have observed that after encystment, metacercariae were without effect (Brown 1926; Hurst 1927), but that during their earlier and active phases, their guts became filled with substances resembling the tissues which they inhabited (Leidy 1850, Hoffman 1899, Krull 1935, Balozet 1937, Dollfus 1938, and Ulmer 1951). The last author identified the gut contents of an unidentified metacercariae as "... kidney tissue."

The present work has shown that the feeding stage larvae cause a general breakdown of the host's kidney tissue, the resultant dêbris being ingested by the parasites, until the kidney is totally destroyed. Although the preceeding caudal stage is apparently benign, there may well be unobserved physiological changes which are not apparent histologically. It has been shown that this feeding of the parasites in the unnatural host A.reticulatus leads to an early mortality among the hosts, the mortality being proportional to the incidence of infection in the population. In the natural host, M.sowerbii, although the renal necrosis occurs to the same extent, the slugs survive for some months, succumbing only after the parasites themselves have reached maturity.

This ability of M.sowerbii to survive without kidney function for so long raises a number of problems; particularly the mechanism whereby survival is effected, and the cause of the ultimate death.

The chief difficulty to be overcome by the slug will be the disposal of waste-substances without kidney-function. In some higher vertebrates this is partially overcome by the use of an alternative mechanism, defæcation. Substances such as allantoin, xanthin, urea, and uric acid appear in the faeces, and are thus eliminated from the body. This is a possibility which should be investigated in slugs.

If waste-products are not eliminated in a manner such as this, they may be stored at some point in the body, possibly the blood. If this is so, it may itself be the ultimate cause of death.

Up to the time of death, the host is not observably different in any way to a healthy individual. It is possible however that excretory-products are accumulating at some point in the body, and that on reaching a certain concentration they lead to the death of the host by way of a toxic action.

These points can be solved only by a biochemical investigation of slug physiology; such an investigation may also show why one species of slug can tolerate for some months conditions which cause rapid death in another species.

Other effects of the metacercariae on the unnatural host, namely reductions in the rate of ingestion of food and of egg-production were observed only under mass-culture conditions, but are nevertheless felt to accurately reflect the state of the individuals within the cultures. The reduction in the incidence of infection during 1953 prevented the hoped-for confirmatory observations on individuals from being made, as well as preventing the investigation of other possible effects. In the present work no other effects were noted, but it is felt that some must be present. The reduction in the rate of feeding will probably affect the weight and growth-rate of the hosts, and this might be investigated, bearing in mind the phenomenon of normal weight changes in slugs, described in section VII. A fuller understanding of the slug physiology however is probably needed before the possible effects of parasitism on the water-relations of the hosts could be confidently investigated. There is obviously a need for biochemical work in both natural and unnatural hosts. It is hoped that investigations similar to those described here will be made on other species of unencysted metacercariae to determine whether the effects recorded here are typical of other such larvae. If they are, an economic significance may emerge, as it may prove possible to control certain pest populations (e.g. A.reticulatus) by artificial infections.

The importance of larval trematodes in this respect has been noted previously, but only as a result of sporocyst and cercarial infections. These are not economically significant owing to the low incidence of infection by such larvae (about 1% for known brachylaemid sporocysts). The present work however has shown that unencysted metacercariae may also exert a lethal effect on their hosts, and that these larvae may parasitize up to 97% of the host population.

Such an infection would be of value if it could be used to control A.reticulatus itself, the commonest slug of Great Britain, and often a serious pest of fields, root-crops, market gardens, and herbaceous borders. If a suitable vertebrate host (e.g. hedgehog) were to be artificially infected and released in the above localities, a metacercarial infection could possibly be spread among the slugs without great difficulty.

It is probable that the present brachylaemid species could not be used for this purpose. Although A.reticulatus is reputed to breed throughout the year, most young individuals are found during April-May, and attain full size in the summer, when they become serious pests. At the time of exposure to brachylaemid infection however (March-April) they were small enough to be immune to parasitization (p.44). Thus although a

number of older individuals will succumb to the infection during the summer, when the "pest-value" is at its peak many individuals will be quite healthy. If a suitable vertebrate host were found, it may of course prove possible to infect it and delay its release until a slightly later date, thus infecting many of the slugs which will be prevalent during the summer. The lethal effect would thus cover many hosts otherwise immune.

A further possibility is that similar effects will be found in other trematode species, the life histories of which do not include a youthful host-immunity to metacercarial infection. This would simplify the question considerably.

A number of difficulties can be foreseen. Man-made interferences with the "balance of nature" can produce unexpected results, and this possibility must be carefully considered. Further, the greatest effects would presumably come from parasites infecting unnatural hosts, and many larval trematodes are notoriously specific in the choice of hosts (Stunkard 1946); this may seriously diminish the number of cases in which an economic control can be effected. Nevertheless the proposal is worthy of investigation in view of the extent of damage due to molluscan pests.

The principal unexplained feature of the present investigation is the reduction in parasite density during 1953. After maintaining a fairly high incidence of infection for at least 4 years, a marked reduction of

parasitism occurred during 1953. Let us consider the two hosts separately.

In A.reticulatus there was a very small incidence of primary infection in March, and the maximum incidence recorded during the year was 20% in October.

During the spring of 1953 no small mammals could be trapped in the infected area, although evidence of them was observed. If these mammals were the vertebrate hosts of the parasites, their disappearance would have led to a natural reduction in the parasite-density. The disappearance of the mammals may have been due to an increase in the cultivation and domestic-life of the area. The fact that infection occurred in M.sowerbii however makes this theory improbable.

The climatic conditions in Feb.-March 1953 were dry and windy, and obviously not favourable to great activity in either vertebrate or invertebrate hosts. Thus it is possible that the slugs were not exposed to infection in the usual manner that year. But is is felt that these conditions could only have slightly reduced the parasite-density, and not virtually extinguished it.

The possibility that the parasite was a "density-dependent factor" is not considered a likely cause of its removal, as there was no corresponding decrease in the A. reticulatus population throughout the year.

Apart from a lowering of the small-mammal population then, no major change occurred in the area to account for the reduction of parasite-density. If any of the above reasons were actually responsible, it emphazizes the dependence of parasites on the vagaries of fauna and climate.

In M.sowerbii no primary infection was recorded until August, after which the incidence rose rapidly and was comparable with that of previous years. It is unknown why the younger stages of metacercariae were not encountered before August, as they must certainly have been present to produce the corrugated and smooth stages seen during the autumn. In view of this fact it is impossible to theorize on the happenings in the natural host population during 1953.

All that can be said with certainty then is that after being marked in some way for a time, the parasite density in M.sowerbii appeared to be normal, but the incidence in A.reticulatus remained low throughout the year.

Ecologically it is interesting to note that a lethal factor such as this parasitism can itself be suddenly and almost entirely lifted from the host-population without any obvious cause. Parasitism as a control-factor in populations is an important ecological principle, but in view of the present observations, the sudden lifting of such a factor may be an equally important principle.

The investigations of the water-relations of slugs were based on the work of Howes and Wells (1934), and although the results of these authors have been confirmed in principle and enlarged, the present work can be regarded only as an introduction to the problem. In future work care must be taken to ensure constancy of experimental conditions; it has already been shown that the normal weight fluctuations were exaggerated by Howes and Wells (1934) owing to the particular conditions within the vivaria.

The reason for the complexity of the water-relations is probably the great amount of water lost by even a slug undergoing no vigorous movement or mucus-production. A.áter under unstimulated conditions lost approximately 33% of initial weight, and Limax flavus about 66% of its initial weight, per 24 hours. (Howes and Wells 1934). These authors also estimated that to maintain a constant level of hydration, a slug would need to drink continuously at a rate equivalent to 2 - 3% of its body-weight per hour, and could thus never move far from a supply of free-water; this figure is for laboratory and not field conditions, where it would often be considerably lower. The presence of the proposed water-retaining mechanism enables the animals to endure alternate phases of hydration and dehydration, and thus survive for short periods without free-water.

In view of the laboratory findings however, slugs obviously live a very restricted life, and must always

have access to free-water. They are active usually under conditions of fairly low temperature and low evaporation rate, and for much of the year free-water will be available in the form of standing rain-water, dew, and plant fluids and exudates. Activity is thus at a time when water-loss is at a minimum, and the chance of imbibing free-water at a maximum. But there must be long periods when conditions are unfavourable. R.H. determinations have shown the layer of air immediately surrounding a slug to be of a high humidity (95 - 100% R.H.) due to evaporation. By retiring into a small pocket in the soil and secreting a cell of mucus, the animal can probably lie indefinitely in a saturated atmosphere, with evaporation reduced to a minimum. Laboratory results however indicate that free-water must be found eventually for survival, although the slowing down of the metabolic rate under these conditions may enable the animals to survive longer than under laboratory conditions.

Fig. 35 showed that A.ater and M.sowerbii of less than about 0.6 gms. weight exhibited drying-out curves even when supplied with drinking-water. The slugs involved were of the same age as larger ones used in other observations and which showed normal weight fluctuations, all having originated from the same batch of eggs. These results may have been a result of the particular conditions within the vivaria, for although a little water was present on the floor as well as in the dish, the

chances of such a small slug encountering the water during its comparatively restricted wanderings were much less than those of a full-sized animal. It is possible then that although water was provided, it was not encountered by these small individuals. The results may however be explainable physically. The smaller animals have a larger surface area/volume ratio than the larger ones and this ratio is further increased by the dorso-ventral flattening in these small individuals. It may be that the water-retaining mechanism (which will presumably be equally as well developed as in the larger individuals of the same age) is unable to cope with the evaporation from the increased surface area/volume ratio. Such slugs, however, live successfully if supplied with a saturated atmosphere as well as drinking-water, and both of these factors may be necessary for development in the field.

Future work on the problem must be directed into two primary channels; a full investigation of slug-physiology, and the application of laboratory results to field conditions.

It has been shown that there are specific differences in the toleration of a lack of drinking water by slugs, and that in some cases a sudden dessication of the animals proves more lethal than one of equal, or greater, severity spread over a longer time.

It would be interesting to know whether the metabolic rate of the animals fluctuates, and whether any

such fluctuations are correlated with those observed in the hydration of the tissues.

Work must be included on the behaviour of various species under field conditions, and correlated with specific differences observed in the laboratory. Dainton (1943) noted reactions of slugs to air currents light, humidity, and temperature, but had obvious difficulty in separating the effects of the latter two. Work of this nature may explain how animals of apparently such a narrow tolerance survive under widely fluctuating field conditions.

APPENDIX I

Activity of Slugs

The time and nature of activity in slugs was investigated by the apparatus illustrated in Fig. 38. In this modified actograph, the rod A was pivoted at the point B and bore the tube C which contained the animal. Each movement of the slug resulted in a change of direction of the record on the smoked drum F. G was a 7-day thermograph, giving a simultaneous recording of temperature. The tube C contained a small pad of moist cotton-wool at its closed end; the other end was covered with muslin. Food could not be given during the course of a recording, but the activity was not affected by whether or not food (lettuce-leaf) was present at the beginning of the experiment.

Specimen recordings are shown in Figs. 39 and 40.

Fig. 39 records the activity of two specimens of A.reticulatus during normal laboratory conditions of alternating light and darkness and fluctuating temperature (16° - 20° C.) and room humidity (50 - 70% R.H.). The white marks on the lower border indicate successive mid-days. The animals remained quiescent during the day, and showed activity for only the first half of the night, the period before dawn being one of rest. The greatest activity was 19.00 - 20.00 hrs. - 03.00 - 04.00. hrs. G.M.T. There was sometimes

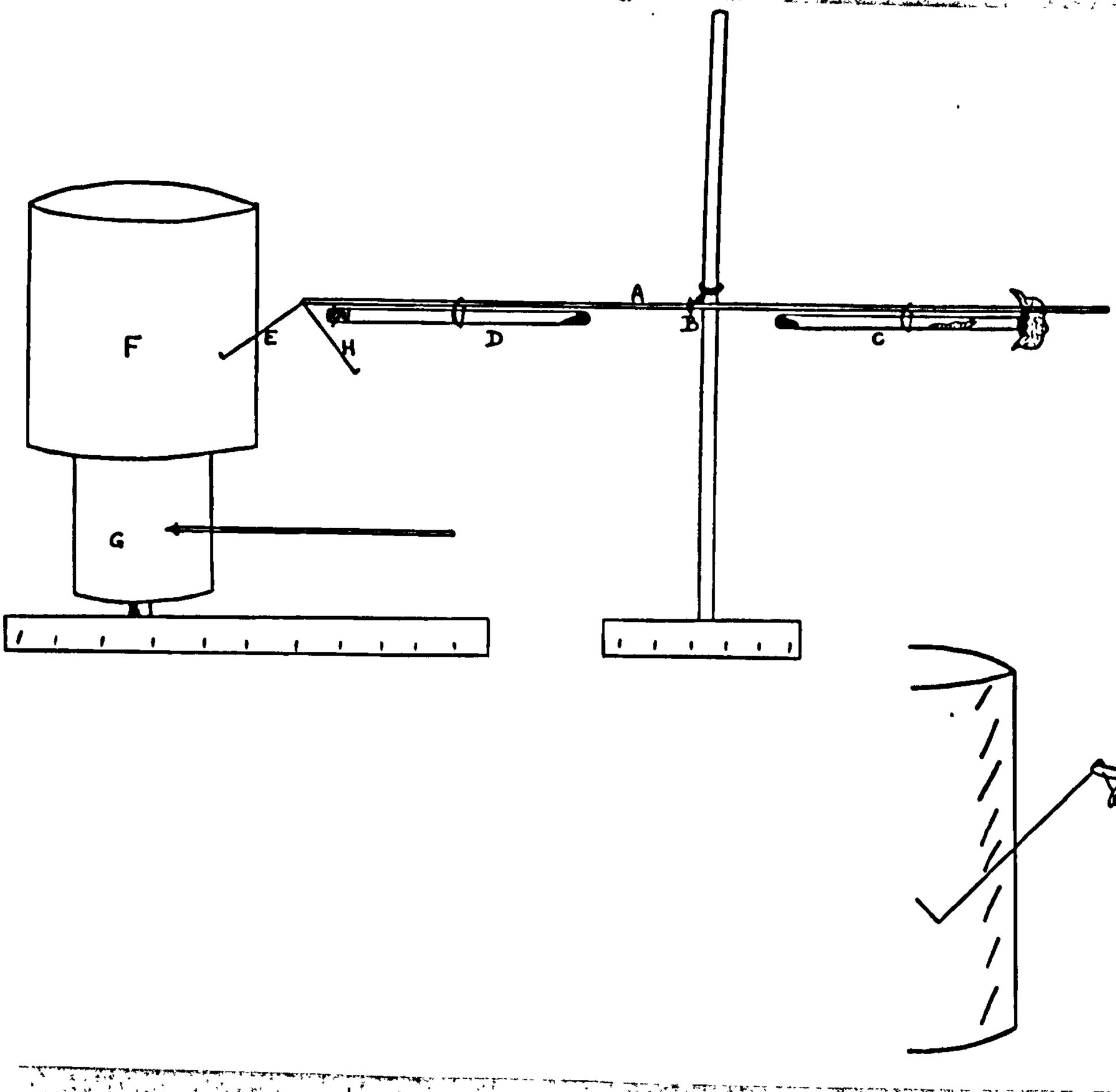


Fig. 38.

Actograph for use with slugs.

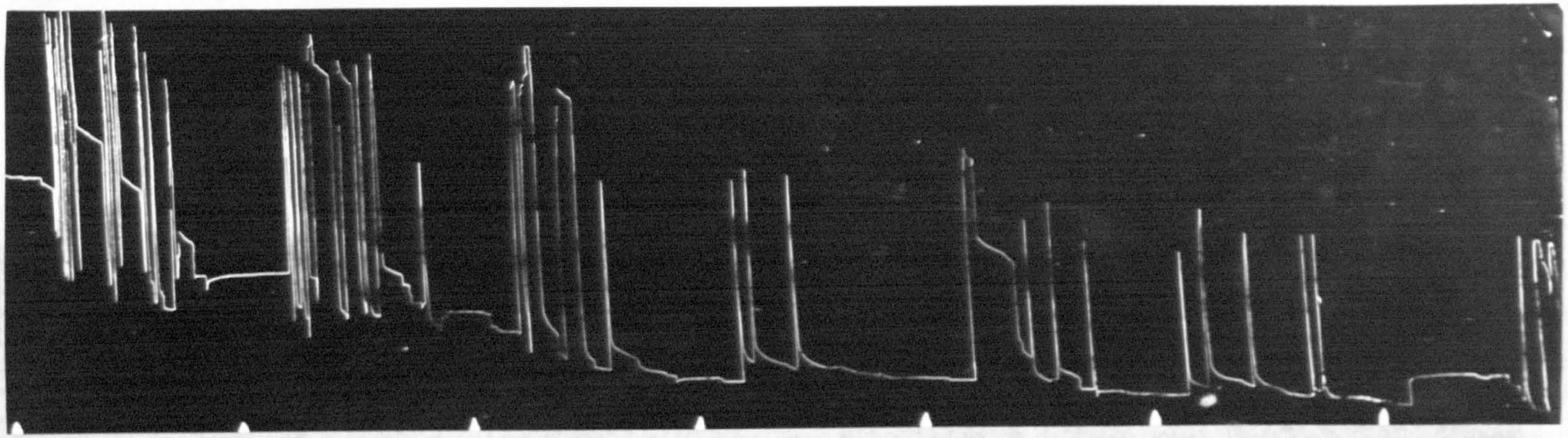


Fig. 39

A.reticulatus. Activity under Fluctuating Conditions

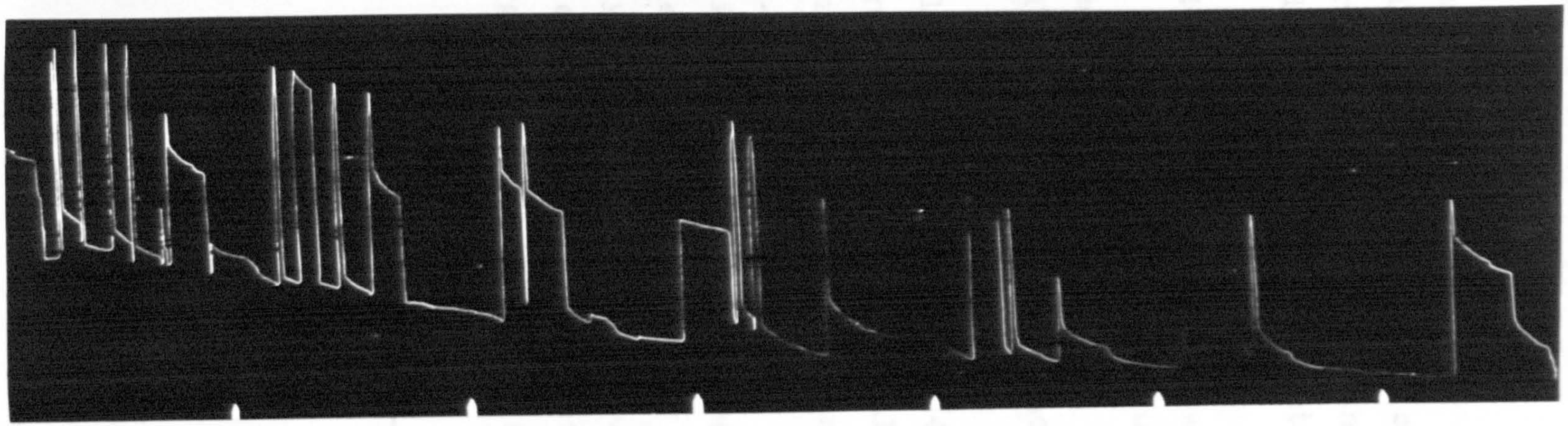


Fig. 40

A.reticulatus. Activity under Conditions of Constant Darkness, Temperature and Humidity.

an unexplained burst of activity about 08.00 hrs.

The slow "dropping" of the complete record resulted from evaporation from the cotton-wool in tube C, and the omission to counterbalance this in tube D.

Fig. 40 shows the activity of a slug under conditions of total and continuous darkness, constant temperature (15°C), and constant humidity (60% R.H.). After the first 48 hours, when movement was prolific and almost continual, the rhythm again became evident, although for a further 48 hours it appeared slightly in advance of the normal timing. After 4 days activity was similar to tnat under varying conditions.

The time of activity in slugs then (i.e. the first half of the night) is apparently not primarily governed by weather conditions or the state of illumination, although the former will undoubtedly play a large part in the extent of movement.

APPENDIX II

Techniques

1. Faecal Examination for Trematode Eggs

(Taylor - personal communication).

1. Shake faeces with water and lead shot until broken down.
2. Pass suspension of faeces through bolting-silk.
3. Centrifuge filtrate at 1500 r.p.m. for one minute.
4. Pour off supernatent liquid, and loosen sediment to form a liquid mud at bottom of centrifuge-tube.
5. Fill tube to within ½inch of top with saturated zinc sulphate solution, and slowly invert a few times until sediment is uniformly mixed with flotation liquid. Avoid air-bubbles.
6. Add drops of saturated zinc sulphate solution to tube until meniscus is convex. Place circular glass cover-slip on top, excluding large air-bubbles.
7. Centrifuge at 1000 r.p.m. for two minutes.
8. Remove cover-slip with a sudden, vertical movement and place on slide for examination.

This is a quantitative method of estimating numbers of eggs of Fasciola hepatica, the number of eggs per

sample being 5/4 x number observed owing to collapse and sinking of some in the zinc sulphate solution.

2. Modified Mallory's Procedure

For slug skin and renal tissue.

1. Embed in paraffin wax or ester wax and section as usual at 7 - 12μ .
2. Sections to water.
3. Acid Fuchsin, 10 secs.
4. 4 rapid changes of water, total 20-30 secs.
5. 1% phosphomolybdic acid, 1 min.
6. Mixture of Aniline Blue and Orange G, 1 min.
7. 4 rapid changes of water, total 10-20 secs.
8. 95% alcohol, 1 min.
9. Alc. Abs., 5 mins.
10. Xylol, 15 mins.
11. Mount in Canada Balsam.

The solution of phosphomolybdic acid and the alcohols should be changed after every 4-5 slides.

3. Hollande Modification of the Courmont-André Method.

(Slightly adapted from Glick 1949).

1. Fix tissues in darkness in fresh solution of equal volumes of 1% silver nitrate and 4.4% formalin, 24 hours.
2. Wash in frequent changes of distilled water, 24 hours.

3. Paraffin sections at 10μ brought to water as usual.
4. Haemalum, 10 mins.
5. Wash in running tap-water, 45 mins.
6. Aqueous Eosin, 45 mins.
7. Wash rapidly in distilled water.
8. 0.5% phosphomolybdic acid, 5 mins.
9. Wash in distilled water.
10. 0.12% aqueous Light Green, 7 mins.
11. 95% alcohol until differentiated.
12. Iso-amyl alcohol, 5 mins.
13. Xylol, 15 mins.
14. Mount in Canada Balsam.

4. <u>Mayer's Mucihaematein, for Mucus Glands in Slugs</u>.

Formula: Haematein 0.2 gms.
Aluminium chloride 0.1 gms.
Glycerine 40 ccs.
Aqua Distil. 60 ccs.

<u>Proceedure</u>:

1. Fix slugs in Bouin's Fluid, 48 hours.
2. Wash in 70% alcohol, 24-48 hours.
3. Dehydrate, clean, and embed normally.
4. Section at 10μ . Sections to water.
5. 10% Mucihaematein, 4 mins.
6. Dehydrate, clean, and mount normally.

Counterstaining (Magenta Red or Picro-Indigo-Carmine) was not employed.

The blue staining of the mucus was the equivalent of a diagnostic histochemical test for the substance.

5. F.A. 410.(Fixative).

Formula: 40% Formalin 10cc.
Glacial Acetic Ac. 10cc.
Water 80cc.

SUMMARY

1. An historical review of the genus Brachylaemus (Dujardin 1843) Blanchard 1847 is given.
2. The history and several proposed classifications of the family Brachylaemidae Joyeux and Foley 1930 is reviewed.
3. A number of brachylaemid life-histories are noted.
4. The literature on the effects of larval trematodes on their hosts is reviewed.
5. Slugs of the species M.sowerbii Férussac and A.reticulatus Müller were found to harbour the metacercariae of an unspecified brachylaemid in kidney.
6. Slugs were not parasitized until a weight of about 150 mgm. was attained.
7. Four distinct stages of metacercarial development are described, and designated caudal stage, feeding stage, corrugated stage, and smooth stage.
8. The caudal- and corrugated stages have a life of about a month, although the latter may last longer.
9. The feeding stage lasts about 6 weeks, and the smooth stage can over-winter in the slug.
10. The mature metacercaria is described; it differed from previously described species.
11. No cyst was present at any time, but the cuticle was thick.

11. The excretory system of the metacercaria is described; flame-cells are considered to be absent.
12. The caudal- and feeding-stage larvae were attached to the kidney lamellae by the ventral sucker.
13. The number of parasites supported by the hosts decreased as one metacercarial stage succeeded another.
14. There was apparently no immunity to re-infection of the slugs.
15. The rate of growth of the metacercariae was constant.
16. A process of decaudation of the caudal stage larvae is proposed.
17. A possible life-history, including a small mammal as the vertebrate host, is proposed.
18. Metacercarial infection of slugs took place in spring, the mature larvae appearing after 4 - 5 months. Infection of the vertebrate host would occur in the autumn.
19. A secondary metacercarial infection of slugs occurred in late autumn.
20. The peak incidence of metacercarial infection was in June-July; the lowest incidence was in Jan.-March.
21. Caudal stage larvae were prevalent in March-May; feeding stage in April-July; corrugated stage in June-October; and smooth stage in August-February.

22. No vertebrate was found to carry a brachylaemid adult.
23. Attempted laboratory infections of mice, guinea-pigs, chickens, and hedgehogs by the oral injection of metacercariae were unsuccessful.
24. The nocturnal activity of slugs was shown to be not primarily governed by climatic conditions.
25. The ecology of the parasite along a ¾ mile stretch of the bank of the River Wear at Durham was investigated.
26. The incidence of brachylaemid infection was consistently higher in M.sowerbii than in A.reticulatus; the former species also proved more susceptible to parasitization.
27. M.sowerbii is concluded to be a natural intermediate host of the parasite.
28. The normal histological structure of the renal organ of slugs is described.
29. Infection by feeding stage larvae led to a general and complete renal necrosis in the hosts, the dêbris being ingested into the gut crura of the parasites.
30. In the unnatural host population A.reticulatus, the renal necrosis led to a host mortality proportional to the incidence of infection in the population.
31. The natural host M.sowerbii withstood the effects of the renal necrosis at least until the parasites had reached maturity.
32. In both host populations the parasitism ultimately acted as a lethal factor.

33. In A.reticulatus the rate of ingestion of food was reduced during infection.
34. In A.reticulatus the rate of egg-production tended to be reduced during infection.
35. Parasitism had no effect on egg-viability, body colouration, general activity, or the histological appearance of organs other than the kidney.
36. Previous (unpublished) work is quoted to show that brachylaemid infection has been known in Durham since 1949.
37. During 1953 there was only a slight incidence of infection among A.reticulatus. No significant fluctuations in the incidence were observed.
38. During August - December 1953 the infection among M.sowerbii was normal, although no caudal- or feeding stage larvae were observed during the spring.
39. No conclusive reason is offered for the peculiarities of the infection during 1953, although it may have been connected with a drop in the mammalian fauna during the winter of 1952/53.
40. The possibility of using unencysted trematode metacercariae to control certain pests is discussed; the inability to use the present species against A.reticulatus is noted.
41. Slugs underwent continual fluctuations of weight under laboratory conditions.

42. Previous authors exaggerated these fluctuations by imposing particular conditions.
43. Water was lost by slugs mainly by evaporation from the skin, and was replaced only by the ingestion of free-water into the crop.
44. Water could not be absorbed through the skin of slugs.
45. In saturated air slugs still lost water but at a reduced rate.
46. A.ater and M.sowerbii of less than about 0.6 gm. weight may have needed both free-water and a saturated atmosphere for life.
47. The presence of a physiological mechanism for the conservation of water in slugs is postulated.
48. Slugs which suffered dessication in low humidity did not recover when returned to saturated air unless they were able to approach and imbibe free-water.
49. A speedy dessication was more detrimental to slugs than one of equal severity spread over a longer period of time.
50. The structure and distribution of the mucus glands of A.ater, M.sowerbii and L.maximus is described.
51. The application of laboratory results to field conditions is discussed, including the need for work on slug behaviour.

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